Original Article

Hereditary Breast-Ovarian Cancer and BRCA1/BRCA2 Variants: A Single Center Experience

Herediter Meme-Over Kanseri ve BRCA1/BRCA2 Varyantları: Tek Merkez Deneyimi

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ABSTRACT

Objective: In this study, it was aimed to determine the frequency of BRCA1 and BRCA2 variants in patients admitted to our clinic with hereditary breast-ovarian cancer and / or family history and to evaluate them in the light of the literature.

Materials and Methods: All patients in our study were selected according to the current NCCN guideline test criteria. The Ion Torrent ™ Oncomine ™ BRCA Research Assay was used to sequence the coding regions of the BRCA1 and BRCA2 genes in our patients. In addition, all patients with copy number changes were confirmed with SALSA® MLPA® Probemix P002 BRCA1 and Probemix P090 BRCA2 (MRC Holland).

Results: Variants (pathogenic, likely pathogenic, variants of uncertain clinical significance, and copy number variations) were detected in 39 of the 149 patients included in the study. Novel variants that were not previously described in the literature were detected in two patients, one of the BRCA1 and one of the BRCA2 gene, respectively.

Conclusion: In our study, the incidence of BRCA1 and BRCA2 variants was found to be 26.1%. This rate was higher than previous studies conducted in Turkey. Further studies are needed to identify common variants in the Turkish population and to evaluate the pathogenity of variants of uncertain clinical significance.

Keywords: Hereditary cancer, breast cancer, ovarian cancer, BRCA1, BRCA2

ÖZET

Amaç: Bu çalışmada kliniğimize herediter meme-over kanseri ve/veya aile öyküsü nedeniyle başvuran hastalardaki BRCA1 ve BRCA2 varyantlarının sıklığının tespiti ve literatür eşliğinde değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmamızdaki tüm hastalar güncel NCCN rehberi test kriterleri doğrultusunda seçilerek dahil edilmiştir. Hastalarımızda BRCA1 ve BRCA2 genlerinin kodlayıcı bölgelerini dizilemek için Ion Torrent™ Oncomine™ BRCA Research Assay kullanılmıştır. Ayrıca kopya sayısı değişiklikleri tespit edilen tüm hastalar SALSA® MLPA® Probemix P002 BRCA1 ve Probemix P090 BRCA2 (MRC Holland) ile konfirme edildi.

Bulgular: Çalışmaya dahil edilen toplam 149 hastanın 39'unda varyantlar (patojenik, muhtemel patojenik, klinik önemi belirsiz varyantlar ve kopya sayısı değişiklikleri) tespit edilmiştir. İki hastamızda (Biri BRCA1 geninde, biri BRCA2 geninde) daha önce literatürde tanımlanmamış yeni varyantlar tespit edilmiştir.

Sonuç: Çalışmamızda BRCA1 ve BRCA2 varyantlarının görülme sıklığı %26,1 olarak belirlendi. Bu oran Türkiye'de yapılan önceki çalışmalara göre daha yüksek bulundu. Türk toplumundaki sık varyantların ve özellikle klinik önemi belirsiz varyantların patojenitesinin daha net değerlendirilebilmesi için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Herediter kanser, meme kanseri, over kanseri, BRCA1, BRCA2

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Introduction

According to the WHO 2020 records, more than 2,250,000 individuals have been diagnosed with breast cancer and the breast cancer has become the most common type of cancer in the world. More than 300,000 women were also diagnosed with ovarian cancer[1]. Many molecular pathways, both genetically and epigenetically, play role in the etiopathogenesis of breast cancer and ovarian cancer. Both cancers show genetic heterogeneity in terms of clinical and biological features. Most cancer cases are considered to be sporadic appearing tumors because there is no clear family history, but cancer syndromes with a known genetic cause or hereditary predisposition to cancer have also been identified [2]. Individuals carrying an inherited genetic mutation and epigenetic abnormalities in tumor suppressor genes have an increased risk of developing cancer throughout their lifetime. Germline mutations in cancer susceptibility genes cause cancer if the normal allele is lost or inactivated. Breast and ovarian cancers (5-10%) can be inherited and occur with cancer-prone syndromes [3].

There are many genes that can increase the risk of developing breast and/or ovarian cancer. In the early 1990s, the BRCA1 and BRCA2 genes were identified as associated with breast and ovarian cancer [4, 5]. Hereditary breast and ovarian cancer (HBOC) caused by pathogenic variants in the BRCA1 and BRCA2 genes is the best known and most common form. It occurs in all ethnic populations. The prevalence of BRCA1/2 pathogenic variants in the population is estimated to be 1/400 to 1/500 [6]. International guidelines such as NCCN state that patients with suspected hereditary breast cancer and all women with epithelial ovarian cancer should seek genetic counseling and comprehensive genetic testing should be recommended. In centers with suitable conditions, patients should be directed to genetic counselors. Before genetic tests, a comprehensive risk assessment should be done to patients and their relatives.

HBOC is a well-known cancer syndrome in which BRCA pathogenic variants are responsible for up to 80% [7]. In addition, high risk gene variants (PALB2, TP53, PTEN) or intermediate risk gene variants (ATM, CHEK2) are also associated with HBOC syndrome [8]. According to a metaanalysis study, individuals with HBOC syndrome have a lifetime risk of developing ovarian cancer (40% for BRCA1 variants and 18% for BRCA2 variants by the age of 70) and/or breast cancer (57% for BRCA1 variants and 49% for BRCA2 variants by the age of 70) [9]. Genetic testing is now widely recommended in cancer diagnosis all around the world and may have the potential to influence treatment decisions. For example, current guidelines recommend the use of poly ADP-Ribose polymerase inhibitors (PARPi) in treatment protocols for patients with BRCA 1/2-related cancer [10]. Therefore, it is very important to determine cancer-related genetic etiology in patients. Identifying pathogenic variant carriers and individuals at risk may reduce morbidity and mortality from cancer. Identifying pathogenic variants in at-risk individuals, it may significantly influence disease course by giving individuals the opportunity to evaluate risk-reducing strategies, such as enhanced surveillance, variant-specific next-generation treatments or surgical interventions.

The National Comprehensive Cancer Network (NCCN) has published recommendations to assist clinicians in identifying individuals with hereditary cancer syndrome, and these recommendations are frequently updated. In the presence of any of the following criteria, there is an indication for genetic testing for hereditary breast, ovarian and pancreatic cancer (Table-1) [11].

After genetic counseling and a comprehensive risk assessment, there may be differences in

Table 1. Testing criteria for breast and/or ovarian cancer susceptibility genes

1.	Individuals with any blood relative with a known Pathogenic/Likely pathogenic variant in a cancer susceptibility gene
2.	Individuals meeting the criteria below but tested negative with previous limited testing (eg, single gene and/or absent deletion duplication analysis) interested in pursuing multigene testing
3.	Personal history of cancer • Breast cancer with at least one of the following ➢ Diagnosed at age ≤45 y; or ➢ Diagnosed at age 46-50 y with ▪ Unknown or limited family history; or ▪ A second breast cancer diagnosed at any age; or ▪ ≥1 close blood relative with breast, ovarian, pancreatic or prostate cancer at any age ➢ Diagnosed at age ≤60 y with triple-negative breast cancer ➢ Diagnosed at any age with; ▪ Ashkenazi Jewish ancestry; or ▪ ≥1 close blood relative with breast cancer at age ≤50 y ovarian, pancreatic, metastatic, intraductal/cribriform histology or high- or very-high-risk group prostate cancer at any age; or ▪ ≥3 total diagnoses of breast cancer in patient and/or close blood relatives ➢ Diagnosed at any age with male breast cancer • Epithelial ovarian cancer (including fallopian tube cancer or peritoneal cancer) at any age • Exocrine pancreatic cancer at any age • Prostate cancer at any age with ➢ metastatic, intraductal/cribriform histology or high- or very-high-risk group ➢ Any NCCN risk group with the following family history: ▪ Ashkenazi Jewish ancestry; or ▪ ≥1 close blood relative with breast cancer at age ≤50 y ovarian, pancreatic, metastatic or intraductal/cribriform prostate cancer at any age; or ▪ ≥2 close relative with either breast or prostate cancer (any grade) at any age • A mutation identified on tumor genomic testing that has clinical implications if also identified in the germline • Individuals who meet Li-Fraumeni syndrome testing criteria or Cowden syndrome/PTEN hamartoma tumor syndrome testing criteria • To aid in systemic therapy decision-making, such as for HER2-negative metastatic breast cancer
4.	Family history of cancer • An affected or unaffected individual with a first- or second-degree blood relative meeting any of the criteria listed above ➢ If the affected relative has pancreatic cancer or prostate cancer, only first-degree relatives should be offered testing unless indicated for other relatives based on additional family history • An affected or unaffected individual who otherwise does not meet the criteria above but has a probability ≥5% of a *BRCA1/2* pathogenic variant based on prior probability models.

choosing the appropriate test for individuals. Preferably, the analysis of BRCA1 and BRCA2 genes, which are known to be associated with hereditary breast and ovarian cancer, is generally a suitable option. However, with the advancing new technologies in recent years, multiple gene panels including BRCA1 and BRCA2 genes can be preferred at the first stage [12].

Material-Method

A total of 149 patients who applied to Başakşehir Çam and Sakura City Hospital, Medical Genetics Department between May 2020 and April 2021 were included in our study. Our study was conducted in accordance with the Declaration of Helsinki and was approved by ethics committee of Basaksehir Çam and Sakura City Hospital (KAEK/ 2021.04.57).

All included patients were selected according to the current NCCN genetic testing criteria in relation to hereditary breast-ovarian cancer syndrome. We examined the variants in the BRCA1 and BRCA2 genes via the next generation sequencing. In addition, we performed CNV analyzes of these genes and also confirmed with MLPA in some of our patients.

Genomic DNA was isolated from peripheral blood of patients after the completion of a consent form. DNA isolations were made from sterile 2 ml EDTA peripheral blood samples using PureLink ™ Genomic DNA Mini Kit. After DNA isolation, the densities of the DNA samples to be included in the study were measured with the Qubit dsDNA HS Assay Kit with the fluorometric method (Qubit® 4.0 Fluorometer), and the final DNA concentrations were ensured to be in the desired range (5-10 ng/µl) for next generation sequencing. Library preparation, BRCA1 and BRCA2 genes were amplified using Oncomine BRCA panel pools using Ion AmpliSeq Library Kit Plus (Life Technologies) in accordance with company protocols. Library products were created in 200 bp fragments. Library products were barcoded with the IonXpress Barcode Adapters Kit (Life Technologies). In order to remove other materials and enzymes from the barcoded samples, the unproduced library products were enzymatically purified by FuPa. At this stage, the application of emulsion PCR and enrichment processes to the normalized library products and the purification and sequencing of the enriched PCRs, the clonal reproduction of the library products containing the target regions by creating oil-water emulsions and loading them on the reading chips were performed on the Ion Chef ™ device. Ion AmpliSeq ™ Library Kit Plus was used for sequencing. In accordance with company protocols; for reading libraries on the Ion GeneStudio S5 Plus sequencer, the enriched products were loaded into the chip (Ion 520 ™ Chip) on the Ion Chef ™ instrument. After sequencing, the BAM files belonging to the data transferred to the Torrent server software were transferred to the Ion Reporter software for analysis. Each patient was analyzed with the programs Ion Reporter™ 5.16.0.3 and The Integrative Genomics Viewer (IGV) with this flowchart. The variants were classified according to the open access databases and ACMG guidelines, and their pathogenicity was determined. Sanger validation was performed for: homo-polymer regions, low quality variants, insertions and/or deletions, splice site alterations and novel variants. Since the reading depths of the samples are at least 300x, CNV (copy number variation) analysis for each sample was also analyzed with Ion Reporter ™ 5.16.0.3 program.

All CNVs detected via the analysis of next generation sequencing data were confirmed by MLPA. SALSA® MLPA® Probemix P002 BRCA1 (MRC Holland) and SALSA® MLPA® Probemix P090 BRCA2 (MRC Holland) were used in combination with a

SALSA MLPA reagent kit according to manufacturer's guidelines. CNV analysis was performed using Coffalyser.Net data analysis software.

Results

A total of 149 patients with breast and / or ovarian cancer or family history selected in line with the NCCN guidelines were included in our study. Variants (Pathogenic, likely pathogenic, VUS and CNVs) in BRCA1 and BRCA2 genes were detected in (26,1%) 39 of 149 patients. While a total of 14 different BRCA1 variants (single nucleotide variations and small indels) were detected in 17 patients, a total of 7 different BRCA2 variants were detected in 8 patients. When these 14 different BRCA1 variants were classified according to the ACMG guideline, 10 of these were considered pathogenic and four of these were considered variant of uncertain significance (VUS). When BRCA2 variants were evaluated according to the ACMG guideline, five of these were considered pathogenic and two of these were considered VUS. To the best of our knowledge, two novel variants were detected which have not been reported in the literature and public databases previously, one in the BRCA1 gene and one in the BRCA2 gene.

When the patients are evaluated according to the application reason, BRCA1 variants were found in 7 of 15 patients and BRCA2 variants were found in 8 of 15 patients tested for breast cancer. 9 patients were tested because of the ovarian cancer. BRCA1 variants were found in 7 of these patients and BRCA2 variants were found in two of these patients. The remaining five patients were evaluated because of their family history of cancer. BRCA1 variants were detected in four and BRCA2 variant in one of these patients.

In addition, as a result of CNV analysis, various deletions and duplications containing one or more exons in the BRCA1 and BRCA2 genes were detected in 11 patients. CNVs were detected in the BRCA1 gene in 7 individuals from five families. Also, CNV was detected in the BRCA2 gene in four individuals from the same family. All of these CNVs were confirmed by MLPA analysis. Detailed information about the patients detected variants are summarized in Table-2 and 3.

Discussion

In the literature, there are many studies conducted in many ethnic groups related to hereditary breast and ovarian cancer and BRCA1/ BRCA2 genes. The rate of detecting genetic variants in these studies varies according to the characteristics of the patients analyzed. For example, in a study of 517 patients in Jordan, pathogenic or likely pathogenic BRCA1 or BRCA2 variants were detected in 72 (13.9%) patients in the whole group in the BRCA1 (n=24, 4.6%) and BRCA2 (n = 48, 9.3%) genes, while VUS was reported in 53 (10.3%) patients [13].

Pathogenic BRCA1/ 2 variants were detected in 13 of 65 patients in a single-center study conducted in Japan in which individuals with triple negative breast cancer were examined. The reason for the small number of patients in the study was emphasized as that the BRCA1/ 2 genetic tests are not under the guarantee of the national health system [14].

There are also several studies conducted in different centers in Turkey associated with the BRCA1 and BRCA2 genes and HBOC recently. In the study of Solmaz et al. published in 2020, variants were detected in 85 of 910 (9.34%) patients selected according to the genetic test criteria in line with the NCCN guidelines. They have determined 31 different variants of the BRCA1 gene in 41 patients and 37 different variants of BRCA2 genes in 44 patients [15]. In another study in published in 2020 conducted in the Thrace region of Turkey, 39 different variants were identified in (17.8%) 88 out of a total of 493

Table 2. *BRCA1* and *BRCA2* sequence variants and pathogenicity classifications of our patients

Patient No	Age	Family History	Reason for application	Gene	Transcript	Location	cDNA change	Protein change	Varyant type	dbSNP	ACMG Classification
1	63	(-)	Ovarian cancer	BRCA1	NM_007300.4	Exon 4	c.181T>G	(p.Cys61Gly)	SNV	rs28897672	Pathogenic (PM1,PM2,PM5,PP3,PP5,BP1)
2	39	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 10	c.1259A>G	p.Asp420Gly	SNV	rs730881442	VUS (PM2)
3	39	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1286T>C	p.Ile429Thr	SNV	rs775869160	VUS (PM2,PM3,BP1)
4	38	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1286T>C	p.Ile429Thr	SNV	rs775869160	VUS (PM2,PM3,BP1)
5	37	(+)	Ovarian cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1504_1508delTTAAA	p.Leu502AlafsTer2	Deletion	rs80357888	Pathogenic (PVS1, PM2, PP3, PP5).
6	48	(-)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.2131_2132delAA	p.Lys711ValfsTer6	Deletion	rs398122653	Pathogenic (PVS1,PM2,PP5)
7	57	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 10	c.2666 C>T	p.Ser889Phe	SNV	rs769712441	VUS (PM2,BP1)
8	39	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.3333delA	p.Glu1112AsnfsTer5	Deletion	rs80357966	Pathogenic (PVS1,PM2,PP5)
9	51	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.3477_3480delAAAG	p.Ile1159MetfsTer50	Deletion	rs80357781	Pathogenic (PVS1,PM2,PP3,PP5)
10	60	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.4036delG	p.Glu1346LysfsTer20	Deletion	rs886040189	Pathogenic (PVS1,PM2,PP3,PP5)
11	24	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 12	c.4246G>C	p.Ala1416Pro	SNV	**Novel**	VUS(PM2,PP3,BP1)
12	72	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 18	c.5159G>A	p.Arg1720Gln	SNV	rs41293459	Pathogenic (PM1,PM2,PM5,PP3,PP5,BP1)
13	43	(+)	Breast Cancer	BRCA1	NM_007300.4	Intron 19	c.5256+1G>A	----------	SNV	rs80358004	Pathogenic (PVS1,PM2,PP3,PP5)
14	47	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
15	42	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
16	45	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
17	45	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 23	c.5507G>A	p.Trp1836Ter	SNV	rs80356962	Pathogenic (PVS1, PM2, PP2, PP3, PP5)
Patient No	**Age**	**Family History**	**Reason for application**	**Gene**	**Transcript**	**Location**	**cDNA change**	**Protein change**	**Varyant type**	**dbSNP**	**ACMG Classification**
1	37	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 5	c.469A>T	p.Lys157Ter	SNV	rs1593886887	Pathogenic (PVS1,PM2,PP3,PP5)
2	81	(-)	Breast Cancer + Pancreatic cancer	BRCA2	NM_000059.4	Exon 11	c.3318C>G	p.Ser1106Arg	SNV	rs1298550035	VUS (PM2,PP3,BP1)
3	49	(-)	Ovarian cancer	BRCA2	NM_000059.4	Exon 11	c.3751dupA	p.Thr1251AsnfsTer14	Insertion	rs397507683	Pathogenic (PVS1,PM2,PP3,PP5)
4	41	(+)	Multiple cancer history in the family	BRCA2	NM_000059.4	Exon 11	c.3751dupA	p.Thr1251AsnfsTer14	Insertion	rs397507683	Pathogenic (PVS1,PM2,PP3,PP5)
5	40	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 11	c.5578A>T	p.Lys1860Ter	SNV	rs431825332	Pathogenic (PVS1,PM2,PP5,BP4)
6	57	(+)	Ovarian cancer	BRCA2	NM_000059.4	Exon 11	c.6054_6058delTAACG	p.Ser2018ArgfsTer29	Deletion	**Novel**	Pathogenic (PVS1,PM2,PP3)
7	31	(-)	Breast Cancer	BRCA2	NM_000059.4	Exon 11	c.6562A>G	p.Lys2188Glu	SNV	rs1135401833	VUS (PM2)
8	33	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 19	c.8395delA	p.Arg2799AspfsTer22	Deletion	rs80359709	Pathogenic (PVS1,PM2,PP5)

Table 3. *BRCA1* and *BRCA2* copy number variations of our patients

Patient No	Age	Famiy History	Reason for application	Gene	Transcript	Exon	CNV type
1	43	(-)	Breast Cancer	BRCA1	NM_007300.4	3-5-6-7-8	Duplication
2	21	(+)	Family history of cancer	BRCA1	NM_007300.4	11	Deletion
3	18	(+)	Family history of cancer	BRCA1	NM_007300.4	11	Deletion
4	54	(-)	Ovarian cancer	BRCA1	NM_007300.4	11	Deletion
5	36	(+)	Family history of cancer	BRCA1	NM_007300.4	18-19	Deletion
6	65	(+)	Breast Cancer	BRCA1	NM_007300.4	18-19	Deletion
7	19	(+)	Family history of cancer	BRCA1	NM_007300.4	24	Deletion
Patient No	**Age**	**Famiy History**	**Reason for application**	**Gene**	**Transcript**	**Exon**	**CNV type**
1	23	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
2	56	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
3	53	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
4	43	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion

individuals selected in line with the NCCN guidelines. The c.5266dupC (p.Gln1756Profs) variant in the BRCA1 gene, which is particularly common in the Ashkenazi population, was identified as the most common variant in this study at a rate of 5.47% [16]. This pathogenic BRCA1 variant was the most common (in three different patients) in our study too. Furthermore, in a large study of 1419 patients from Turkey in 2020, pathogenic variants were identified in (9.4%) 134 patients and likely pathogenic variants in (0.3%) five patients. BRCA1 variants were detected in 58 of these patients and BRCA2 variants were detected in 64 of them. Also, variants of uncertain significance were detected in (6.4%) 91 patients [17]. Additionally, less than breast and ovarian cancers, there is also an increased risk of developing other types of cancer at solid organs including prostate cancer, melanoma, and pancreatic cancer. Recent years, BRCA variant spectrum of pancreatic cancer has been determined both nationally and worldwide [18, 19].

In our study, the incidence of BRCA1 and BRCA2 variants in our cohort was 26.1%, which was higher than in previous studies conducted in Turkey. This result may be due to relatively lower number of patients in our study. It may have also resulted from the fact that a more well-selected group was tested.

When the distribution of variants on the gene was examined, it was seen that the variants in the BRCA1 gene were mostly (9/18, 50%) on the 10th exon. It was observed that the variants in the BRCA2 gene were mostly (6/9, 66%) on the 11th exon. When the literature, ClinVar and HGMD were examined, it was seen that these regions were hot-spot regions for these genes.

According to the literature, some BRCA1 and BRCA2 variants are common in certain populations. For example; 3 different variants (BRCA1 c.68_69delAG, BRCA1 c.5266dupC and BRCA2 c.5946delT) are seen in the majority of cases in Ashkenazi Jewish patients, which is also a testing criterion in the NCCN guidelines. When we look at the studies conducted in Turkish society, we see that the variants do not cluster but generally show a distribution. We also detected one novel variant each in BRCA1 and BRCA2

genes. The patient with a novel variant in the BRCA1 gene was a female diagnosed with breast cancer at 24 years old. Her family history was unremarkable. The detected BRCA1 variant (NM_007300.4:c.4246G>C: p.Ala1416Pro) was evaluated as uncertain clinical significance (VUS) according to the ACMG guidelines. The other patient, in whom we detected a novel variant in the BRCA2 gene, was admitted with the diagnosis of ovarian cancer at the age of 57. In the family story, her sister had ovarian cancer and her grandmother had a history of breast cancer. The detected BRCA2 variant (NM_000059.4:c.6054_6058del:p.Ser2018ArgfsTer29) was evaluated as pathogenic according to the ACMG guidelines.

Large deletions / duplications in the BRCA1 and BRCA2 genes differ between populations. It is estimated to be around 10 percent on average. Methods such as MLPA are used to detect large CNVs in the BRCA1 and BRCA2 genes. However, the multistep approach with CNV analysis followed by sequencing may be both expensive and time consuming. Recent developments with the NGS technology now allow simultaneous detection of CNVs and single nucleotide variations/small indels using different bioinformatic pipelines. We used Oncomine™ BRCA assay in combination with Ion Reporter ™ 5.16.0.3 software for this purpose. CNVs were found in %7.3 of cases in our cohort and all cases were confirmed by MLPA. Germani et. al (2018) reported %100 concordance between NGS results and MLPA/multiple amplicon quantification (MAQ) results using the same approach [20]. In another study, various assays analyzed with Sophia DDM platform and SeqNext software were compared with conventional methods [21]. Sensitivity of BRCA Tumor and BRCA HC assays analyzed with both Sophia DDM platform and SeqNext software were reported 100%. The specificity was highest (100%) for BRCA HC Assay-Sophia DDM platform combination and lowest (99.489%) for BRCA HC assay-SeqNext software combination. These data suggest that NGS-CNV detection algorithms show promise for a more efficient approach instead of multi-step testing. However, it is important to validate the assays and bioinformatic pipelines.

Conclusion

In this study, our experience of one year in our center is summarized in the light of scientific literature. We detected two novel variants in our cohort that were not previously described in the literature. For patients whose BRCA1 and BRCA2 variants could not be detected and who meet the NCCN hereditary breast and ovarian cancer syndrome criteria, multigene panels have been recommended to examine additional genetic causes. Further studies are needed in our country in order to evaluate the pathogenicity of BRCA1 and BRCA2 genes, especially variants of uncertain clinical significance.

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Original Article

Molecular Characterization Reveals the Importance and Diversity of Germline and Somatic RET Mutations in Cancer

Moleküler Karakterizasyonla Tespit Edilen Germline ve Somatik RET Mutasyonlarının Kanserdeki Önemi ve Çeşitliliği

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ABSTRACT

Aim: Many individuals die due to cancer, and both doctors and researchers work hard to offer accurate illness, diagnosis, and prognosis monitoring, as well as resistance prediction.
Methods: A liquid biopsy and hereditary cancer panels were performed on 25 patients to examine the importance, spectrum, and diversity of RET germline and somatic mutations. Most of the patients visited the clinic with the diagnosis of advanced resistant cancers or hereditary cancer (MEN2). Two groups were formed: the first group was germline (n=7, 28%), and the second was somatic (n=18, 72%). For somatic, Tier I-II-III variants; for germline, pathogenic, likely pathogenic, and VUS variants have been included in the study.
Results: The mean age was 54.64. There were significantly more female participants (n=14, 56%) than males (n=11, 44%). In the germline group, the most common mutation was 'RET:c.2410G>A'. Nine mutations were nonsense or frameshift in the somatic group, and the most common mutations were 'RET:c.2324delinsGAC' and 'RET:c.1784A>G'. Nonsense or frameshift RET variants showed a higher incidence in the somatic group.
Conclusion: To the best of our knowledge, this is the first research to concentrate on RET mutations in the context of genetic variability between germline and somatic variants. The current of the study results indicate that patients with solid tumors, particularly breast cancer, should undergo RET sequencing to evaluate clinical features and prognosis. Discoveries about the structure and functions of RET gene will lead to more clinically relevant treatment approaches for cancer patients and will play an essential role in improving individual risk prediction, treatment, and prognosis.

Keywords: Liquid biopsy, MEN2, RET

ÖZET

Amaç: Pek çok kişi kanser nedeniyle ölmekte. Hem doktorlar hem de araştırmacılar, doğru hastalık, teşhis ve prognoz takibinin yanı sıra direnç tahmini sunmak için çok çalışıyorlar.
Gereç ve Yöntem: RET germline ve somatik mutasyonların önemini, spektrumunu ve farkını incelemek için 25 hastaya likit biyopsi ve ailesel kanser paneli uygulandı. Hastaların çoğu ileri dirençli kanser ve / veya kalıtsal kanser (MEN2) tanısıyla kliniği ziyaret etti. Toplam iki grup oluşturuldu: birinci grup germline (n=7, %28) ve ikincisi somatik (n= 8, %72). Somatik için, Tier I-II-III varyantları ve germline için patojenik, muhtemelen patojenik ve VUS varyantları çalışmaya dahil edilmiştir.
Bulgular: Ortalama yaş 54.64 idi. Kadın katılımcılar (n=14, %56) erkeklerden (n=11, %44) önemli ölçüde daha fazla idi. Germline grubunda en yaygın mutasyon "RET: c.2410G>A" idi. Somatik grupta, dokuz mutasyon nonsense veya çerçeve kaymasıydı ve en yaygın mutasyonlar "RET: c.2324delinsGAC" ve "RET: c.1784A>G" idi. Nonsense veya çerçeve kayması RET varyantları, somatik grupta daha yüksek bir insidans gösterdi.

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Sonuç: Bildiğimiz kadarıyla bu, germline ve somatik varyantlar arasındaki genetik değişkenlik bağlamında RET mutasyonlarına odaklanan ilk araştırmadır. Mevcut çalışmanın sonuçları, solid tümörlü hastaların, özellikle meme kanserinin, klinik özellikleri ve prognozu değerlendirmek için RET sekansına tabi tutulması gerektiğini göstermektedir. RET geninin yapısı ve işlevleri hakkındaki keşifler, kanser hastaları için klinik olarak daha uygun tedavi yaklaşımlarına yol açacak ve bireysel risk tahmini, tedavisi ve prognozunun iyileştirilmesinde önemli bir rol oynayacaktır.

Anahtar Kelimeler: Likit biyopsi, MEN2, RET

Introduction

Receptor tyrosine kinases regulate cell development and differentiation. Some of them have been shown to behave as oncogenes in human malignancies. RET (rearranged during transfection) is a trans-membrane receptor tyrosine kinase that may act as both a growth factor receptor and an oncogenic protein. It is triggered by a complex that includes a soluble glial cell line-derived neurotrophic factor (GDNF) family ligand (GFL) and a glycosylphospha-tidylinositol-anchored co-receptor, GDNF family receptors a (GFRa) [1]. GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN) are four distinct GFLs that can bind to and selectively activate RET through their homologous co-receptors GFRa1–4. RET has multiple activities in diverse tissues as a signal transducer of four separate ligand/co-receptor complexes. It is required for the development of the enteric nervous system as well as the regulation of the development of sympathetic, parasympa-thetic, motor, and sensory neurons [2].

The RET protein is a receptor tyrosine kinase that seems to transduce growth and differentiation signals in a variety of developmental tissues, including neural crest-derived tissues. The protein comprises an extracellular domain containing a ligand-binding domain, a cadherin-like domain, and a cysteine-rich region proximal to the cell membrane. It includes one transmembrane domain and two tyrosine kinase subdomains, TK1 and TK2 [3].

Somatic and germline mutations in the same tumor suppressor gene are widely known, as detailed in Knudson's two-mutation paradigm [4]. Similarly, somatic and germline mutations in the RET protooncogene have been discovered in a number of hereditary and non-hereditary human disorders, including multiple endocrine neoplasia (MEN) 2A and 2B, papillary thyroid cancer, and other cancers [5].

Multiple endocrine neoplasia type 2 (MEN2), sometimes referred to as Sipple's syndrome, is linked with medullary thyroid carcinoma (MTC) and hyperplasia of thyroid C cells. It is an autosomal dominant genetic disorder caused by a mutation in the RET proto-oncogene on chromosome 10, which results in the development of two or more endocrine adenomas or hyperplasia in the same patient, either simultaneously or sequentially, and resulting in the clinical condition defined by hyperfunctioning glands [6].

MEN2 is classified clinically as MEN2A, MEN2B, and familial medullary thyroid cancer, with MEN2A being the most frequent subtype [1]. Medullary thyroid cancer (MTC), pheochromocytoma (PHEO), and hyper-parathyroidism are all characteristics of MEN2A. Additionally, a tiny percentage of people develop skin lichen amyloidosis or Hirschsprung's disease. MTC is often the initial symptom of this subtype, with a near-100 percent prevalence. When patients are hospitalized, the majority have already advanced to MTC or have lymph node metastases. MTC is the leading cause of mortality in people with MEN2A, and 50% of

patients are at risk of recurrence [7]. MTC or MEN2A, on the other hand, may manifest differently in family members. Specifically, fundamental lesions may be entirely or partially manifested, lesions in the affected endocrine glands may arise at various time intervals (which may be many years), and numerous endocrine glands may sometimes be affected and demonstrate concurrent start. At the moment, individuals with MEN2A who demonstrate MTC as an early symptom are often misdiagnosed [1].

Numerous malignancies are known to be oncogene-dependent: oncogene addiction has been shown in a variety of neoplasms [8]. Somatic RET gene fusions are known to be oncogenic drivers in a variety of tumor types and are seen in 1–2% of non-squamous NSCLC patients. Fusions of the RET gene result in the formation of chimeric, cytosolic proteins containing a constitutively active RET kinase domain [9]. The recent approval of numerous tumor-agnostic medications by the Food and Drug Administration has resulted in a paradigm shift in cancer therapy away from organ/histology-specific strategies and toward biomarker-guided treatments. Selpercatinib (LOXO-292), a novel RET-specific tyrosine kinase inhibitor, has shown exceptional effectiveness in cancers with RET fusions or mutations, most notably RET fusion-positive NSCLC and RET-mutated MTC [10].

Liquid biopsy techniques have been used to treat a variety of different forms of cancer in recent years. A liquid biopsy is utilized in tumors to determine the patient's recovery, prognosis, and even diagnosis. During apoptosis, tumor cells lose fragments of biomarkers. These materials' cellular components may be examined for genetic abnormalities. This less intrusive testing procedure provides a greater likelihood of a favorable outcome and a better probability of correct findings [11,12].

In this study, we performed a liquid biopsy and hereditary cancer panel on 25 patients to examine the importance, spectrum, and difference of germline and somatic RET mutations. Our data broadens the RET mutations and provides insights for the diversity and characteristics of somatic and germline RET mutations.

Materials and methods

Patients

Consent for the publication of the study and any additional related information was taken from the patients or their parents involved in the study. The Ethics Committee approved (2021-03/1072) the study at the University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital. Twenty-five patients visited the clinic with the diagnosis of advanced resistant cancers or hereditary cancer (MEN2). Clinical histories and molecular results were reviewed for all unrelated patients examined at the Department of Medical Genetics, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, and Department of Medical Genetics, University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Ankara, Turkey. The patients underwent the comprehensive liquid biopsy and hereditary cancer panel between January 2018 and December 2020 at the Ankara Central Genetic Laboratory (Turkey). In the study, a total of two groups were formed. The first group was germline (n=7, 28%) and the second was somatic (n=18, 72%).

DNA Panels and NGS

From the blood samples collected in EDTA tubes, the patients' genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen Inc., Hilden, Germany) by QIAcube (Qiagen Inc.,

Mississauga, ON, Canada). The DNA samples were quantified with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA).

Two different multigene panels have been used for liquid biopsy testing depending on the dates: ArcherDx Reveal ctDNA 28 Kit and Sophia Genetics 56 G Oncology. The Sophia Genetics 56G Oncology Solution was used at the center from 2018 to 2020, and the ArcherDx Reveal ctDNA 28 Kit has been used since 2020. The data were analyzed on the Archer Analysis Platform (ArcherDX, Inc., CO, USA) for the ArcherDx Reveal ctDNA 28 Kit and Sophia DDM software (Sophia Genetics, Saint-Sulp) for the Sophia Genetics 56G Oncology Solution.

For hereditary cancers, two different multigene panels were used depending on the dates: the Qiagen QIAseq Hereditary Custom Cancer Panel (from 2017 to 2018) and the Sophia Hereditary Cancer Solution Panel (since 2018). The sequencing was performed on an Illumina MiSeq system (Illumina Inc., San Diego, CA, USA). The data were analyzed using QIAGEN Clinical Insight (QCI™) Analyze software (Qiagen Inc., Hilden, Germany) for the Qiagen QIAseq Hereditary Custom Cancer Panel and with Sophia DDM software (Sophia Genetics, Saint-Sulp) for the Hereditary Cancer Solution (v1.1) panel. Visualization of the data was performed with IGV 2.7.2 (Broad Institute) software.

Interpretations, Descriptive Statistics & Graphics

In compliance with the recommendations issued by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, germline variants were categorized as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign [13]. Pathogenic, likely pathogenic, and strong VUS (supports clinical phenotype and no other responsible mutation detected) variations were included in the study. Somatic variants were categorized as tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants with unknown clinical significance; and tier IV, variants that are benign or likely benign, in compliance with the recommendations issued by the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists [14]. Tier I-II-III variations have been included in the study. Further, descriptive statistical calculations have been done, and the graphic has been prepared with Python 3.9.2 (IPython 7.19.0).

Results

The mean age was 54.64, with a minimum age of 35 and a maximum of 70. There were six patients below 50 years of age, and all of them were females. There were significantly more female participants (n=14, 56%) than males (n=11, 44%) (Table 1-2).

In the germline group, the mean age was 50.57, and all the mutations were missense and heterozygous. There were three pathogenic, two likely pathogenic, and two variant of uncertain significance (VUS) variants. The most common mutation was ‘RET:c.2410G>A’ (Table 1, Figure 1).

In the somatic group, the mean age was 56.22, and the variant fractions were between 0.1-10%. The majority of the patients have advanced-metastatic cancers. Nine mutations were nonsense or frameshift. The most common mutations detected were ‘RET:c.2324delinsGAC’ and ‘RET:c.1784 A>G’. The ‘RET:c.2324delinsGAC’ mutation has been observed seven times. (Figüre 1) In breast cancer, frameshift RET mutations were more predominant when compared with other groups (Table 2).

Table 1. RET germline mutations

Gender	Age	Indication	Gene	Mutation	Protein	Zygosity	Pathogenicity
F	53	colon	RET	c.1681A>T	p.Ser561Cys	heterozygous	Likely Pathogenic
F	35	MEN2	RET	c.224C>T	p. Thr75Met	heterozygous	Likely Pathogenic
F	41	MEN2	RET	c.785T>C	p.Val262Ala	heterozygous	VUS
M	67	MEN2	RET	c.341G>A	p.Arg114His	heterozygous	VUS
M	52	MEN2	RET	c.2370G>T	p.Leu790Phe	heterozygous	Pathogenic
F	56	MEN2	RET	c.2410G>A	p.Val804Met	heterozygous	Pathogenic
M	50	MEN2	RET	c.2410G>A	p.Val804Met	heterozygous	Pathogenic

Table 2. RET somatic mutations

Gender	Age	Indication	Gene	Mutation	Protein
F	53	advanced-metastatic	RET	c.1162G>A	p.Val388Ile
M	58	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
M	61	advanced-metastatic	RET	c.2071G>A	p.Gly691Ser
F	59	advanced-metastatic	RET	c.2372A>T	p.Tyr791Phe
M	66	advanced-metastatic	RET	c.1972C>T	p.His658Tyr
M	60	advanced-metastatic	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	48	breast	RET	c.1906A>C	p.Thr636Pro
M	62	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
M	51	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
F	37	breast	RET	c.2338_2339insC	p.Lys780Thrfs*64
F	69	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	46	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
M	57	advanced-metastatic	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	55	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	37	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
M	58	advanced-metastatic	RET	c.2341C>T	p. Gln781Ter
F	70	lung	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	65	advanced-metastatic	RET	c.2657G>A	p.Arg886Gln

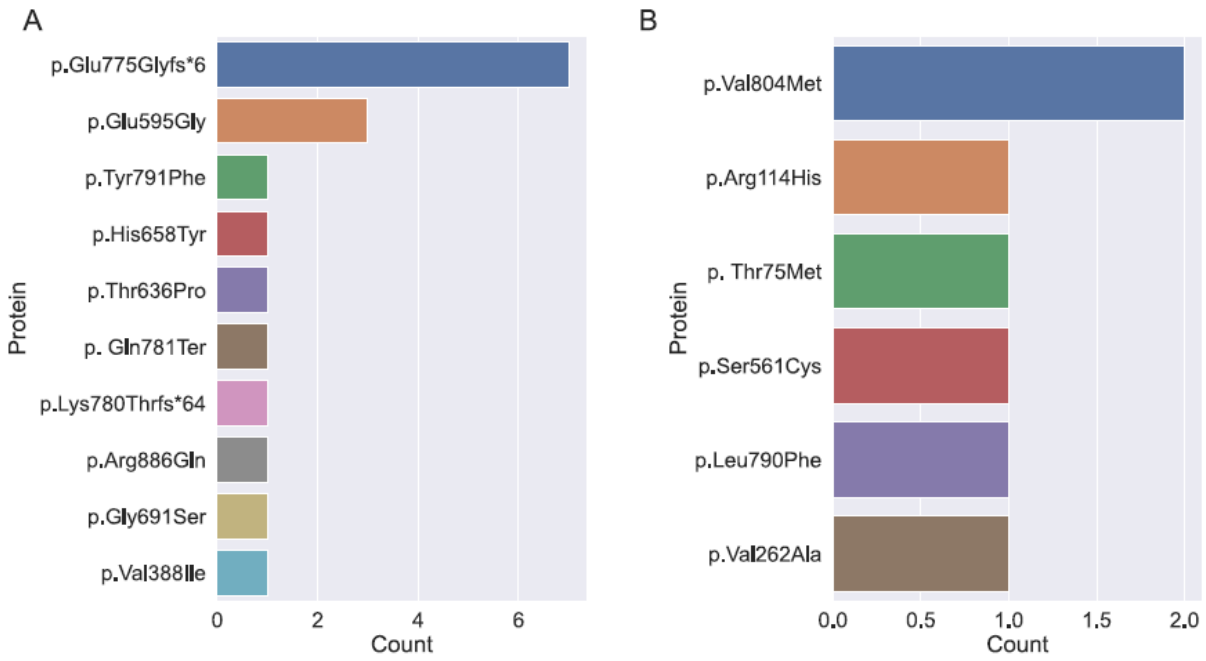

Figure 1. Somatic and germline RET mutations.
Bar plots showing the somatic (A) and germline (B) RET mutations in the study.

Discussion

Mutations in the RET gene result in various clinical symptoms and disease manifestations [2]. Based on RET's normal function, it is conceivable to identify various probable explanations for the disparate phenotypes. The signaling capability of various RET variants may be determined by subcellular location, substrate selectivity, turnover rate, percentage of activated RET, and genetic background. As a result, distinct types of clinical symptoms associated with RET may need treatment with different sorts of medications targeting specific domains of RET [2].

While germline mutations in codons 768 (exon 13), 804 (exon 14), and 891 (exon 15) are strongly related to MTC, they account for a small proportion of cases. These locations are located inside the domain of the intracellular tyrosine kinase. Exon 13 mutations are less prevalent in MEN2A/MTC (codons 790 and 791). Gatekeeper mutations in codon 804 have been found. Codon 804 mutation was found in two patients in the germline group in this study. (Figure 1) Changes at this location affect access to the RET ATP-binding domain, resulting in decreased sensitivity to some RET-targeting multi-kinase inhibitors [15]. Mutations in the intracellular TK2 domain are responsible for MEN2B-associated malignancies. A single 918 Met to Thr mutation in exon 16 accounts for almost 95% of MEN2B cases and is unique to this illness. Met 918 is a crucial component of the substrate recognition pocket found in the RET protein's tyrosine kinase catalytic core. Mutations arise as new (de novo) germline alterations in more than 50% of cases of MEN2B with codon 918 mutations. Another mutation, alanine to phenylalanine at codon 883 in exon 15, was discovered in some unrelated MEN2B relatives [16]. Dual (tandem) mutations in codons 804 and 806 or 804 and 904 may result in atypical MEN2B [17].

MEN2 RET mutations in the germline result in a gain of function. This contrasts with many other hereditary predispositions to neoplasia, which is caused by heritable "loss-of-function" mutations in tumor suppressor proteins. The functional restrictions imposed by such activating lesions are likely responsible for the rarity of RET mutations, a regulation that benefits molecular diagnostics in this condition [18].

Extensive research on large families demonstrates a clear genotype-phenotype link. MEN2B has a higher rate of morbidity and death than MEN2A. Survival is comparable between individuals with MEN2B and those with spontaneous MTC who had somatic RET mutations identical to the most prevalent germline mutations causing MEN2B. The genotype also affects the age at which MTC is first diagnosed and the result of thyroidectomy [19].

RET gene rearrangements are essential for solid tumors. In this study, nonsense and frameshift RET mutations were frequent in the somatic group, particularly breast cancer. 'RET, c.2324delinsGAC, p.Glu775Glyfs* 6' mutation was the most common. (Table 2, Figure 1) All the nonsense and frameshift RET mutations were on the 13th exon and in the kinase domain. The majority of the somatic group mutations were around the kinase domain. Most of the kinase domain RET mutations are oncogenic and associated with poor prognosis and drug resistance, particularly in thyroid cancers [20].

In contrast to the germline group, frameshift and kinase domain RET mutations were predominant in the somatic group. Many nonsense and frameshift RET mutations are also associated with gain of function according to databases (OncoKB), and they are likely oncogenic, unlike other genes. These mutations, particularly 'RET, c.2324delinsGAC, p.Glu775Glyfs*6', could be responsible for drug resistance,

progression, and metastasis. Further studies are needed to clarify the roles of these nonsense and frameshift RET mutations.

The current study's results indicate that patients with solid tumors, particularly advanced-metastatic cancers and breast cancer, should undergo RET sequencing to evaluate clinical features and prognosis. Discoveries about the structure and functions of RET gene will lead to more clinically relevant treatment approaches for cancer patients and will play an essential role in improving individual risk prediction, treatment, and prognosis.

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Original Article

Real-Life Analysis of Immunotherapy as the Second or Later Lines Treatment in Patients with Metastatic Non-Small Cell Lung Cancer

Metastatik Küçük Hücre Dışı Akciğer Kanseri Hastalarının İkinci veya İleri Sıra Tedavisinde İmmünoterapinin Gerçek Yaşam Analizi

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ABSTRACT

Background: Immunotherapy agents such as atezolizumab and nivolumab are appropriate option for non-small cell lung cancer (NSCLC) accounts in the absence of driver mutation, regardless of PDL-1 expression in second and later line setting. Herein we aimed to evaluate the efficacy and safety of immunotherapy for the second and later line settings in metastatic NSCLC patients as a single center experience.

Methods: Totally, 37 patients with metastatic NSCLC who received atezolizumab or nivolumab in the second or later lines were included. Clinicopathological features of patients and survival outcomes were analyzed. The safety profile and the factors that may predict survival were also evaluated.

Results: Twenty-nine (78.4%) of patients were men and 8 of patients (21.6%) were woman with median age of 61 years (range:42-80). Atezolizumab was preferred in 22 (59.5%) of these patients and nivolumab in 15 (40.5%) of them. Objective response rate was 35.1%. At a median follow up of 22.5 months, median progression-free survival (PFS) was 4.7 months, median overall survival (OS) was 24.1 months. Univariate analysis for PFS revealed that gender (p=0.03), age (p=0.005), the presence of brain metastasis (p=0.02), PDL-1 status >1% (p=0.035), ECOG PS (p=0.04) and the good response to frontline treatment (p=0.015) were found to be significant prognostic indicators. It also showed that the presence of brain metastasis (p=0.03), PDL-1 status >1% (p=0.027), good response to firstline treatment (p=0.022) and atezolizumab preference (p=0.018) were prognostic factors for OS.

Conclusion: Our real-life analysis indicated that atezolizumab and nivolumab improved survivals with good safety profile in second and later lines treatment of metastatic NSCLC patients.

Keywords: Non small cell lung cancer, atezolizumab, nivolumab, second or later line treatment

ÖZET

Amaç: Atezolizumab ve nivolumab, driver mutasyon yokluğunda, küçük hücre dışı akciğer kanserinin (KHDAK) ikinci ve sonraki basamak tedavisinde PDL-1 durumundan bağımsız olarak kullanılabilen iyi bir seçenektir. Burada, metastatik KHDAK'li hastalarda ikinci ve sonraki sıra tedavide immünoterapinin etkinliğini ve güvenliğini değerlendirmeyi tek merkez deneyimi olarak amaçladık.

Gereç ve yöntem: Çalışmaya, ikinci veya sonraki sıralarda atezolizumab veya nivolumab alan toplam 37 metastatik KHDAK hastası dahil edildi. Hastaların klinikopatolojik özellikleri ve sağkalım sonuçları analiz edildi. Güvenlik profili ve sağkalımı öngörebilecek faktörler değerlendirildi.

Bulgular: Hastaların 29'u (%78.4) erkek, 8'i (% 21.6) kadın, ortanca yaş 61 (aralık: 42-80) idi. Bu hastaların 22'sinde (%59.5) atezolizumab, 15'inde (% 40.5) nivolumab tercih edilmişdi. Objektif yanıt oranı %35.1 idi. Medyan 22.5 aylık takipte, medyan progresyonsuz sağkalım 4.7 (PSK) ay iken, medyan genel sağkalım (OS) 24.1 ay olarak bulundu. PFS için tek değişkenli analizde, cinsiyet (p=0.03), yaş (p=0.005), beyin metastazı varlığı (p=0.02), PDL-1 durumu >%1 (p=0.035), ECOG PS (p=0.04) ve ilk sıra tedaviye iyi yanıt varlığı (p=0.015) anlamlı prognostik göstergeler olarak bulundu. OS için ise, beyin metastazı varlığı (p=0.03), PDL-1 durumu >%1 (p=0.027), ilk sıra tedaviye iyi yanıt varlığı (p=0.022) ve atezolizumab tercihi (p=0.018) prognostik faktörler olarak bulundu.

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Sonuçlar: Gerçek hayat analizimiz, atezolizumab ve nivolumabın, metastatik KHDAK hastalarının ikinci ve sonraki basamak tedavilerinde iyi güvenlik profili ile sağkalımı iyileştirdiğini gösterdi.

Anahtar Kelimeler: Küçük hücre dışı akciğer kanseri, nivolumab, atezolizumab, ikinci ve sonraki sıra tedavi

Introduction

Lung cancer is the mostly diagnosed cancer worldwide and causes deaths approximately 1.7 million per year [1]. Non small cell lung cancer (NSCLC) is about 80% of lung cancers. Half of patients are diagnosed in the advanced setting, however survival rates are improving in recently years due to new treatment modalities [2]. Targeted therapies are appropriate option with presence of driver mutation e.g., epidermal growth factor receptor [EGFR]-mutant, anaplastic lymphoma kinase [ALK]-rearranged NSCLC. Nevertheless, in those with the lack of driver mutation immune check point inhibitors with or without chemotherapy is the best treatment option which has led to improvements in survival and quality of life [3]. Although immunotherapy is preferred at initial treatment setting, many patients are treated with frontline chemotherapy. For such patients regardless of PDL-1 expression status, anti-programmed cell death protein 1 (PD-1) or anti-programmed cell death ligand 1 (PDL-1) antibody is an appropriate option rather than single agent chemotherapy. Unlike atezolizumab and nivolumab, pembrolizumab is an option if the tumor PDL-1 has been identified in at least 1% of tumor cells [4-6]

Nivolumab, with the dose of 240 mg IV every two weeks, is an option for advanced NSCLC patients who progressed after platinum-based chemotherapy. In the phase III CheckMate 017 trial nivolumab compared with chemotherapy in squamous NSCLC and nivolumab improved overall survival (OS) with median 9.2 versus 6.0 months. PDL-1 status did not change the survival rates [7-9]. In the phase III CheckMate 057 trial, nivolumab was compared with docetaxel in advanced non squamous NSCLC, nivolumab also prolonged OS with median 12.2 versus 9.4 months. However, survival improvement was seen in PDL-1 positive tumors, which was similar between nivolumab and docetaxel for those with PDL-1-negative tumors [10,11].

Atezolizumab was approved for dose schedule 1200 mg IV every three weeks. In phase III OAK trial atezolizumab compared with docetaxel in advanced pretreated NSCLC with any PDL-1 and histologic status. Atezolizumab experienced improved OS, 13.8 versus 9.6 months regardless of histology. Atezolizumab versus docetaxel did not improve the PFS or response rates. Also higher PDL-1 status was related with greater OS results [12,13].

Pembrolizumab with approved dose of 200 mg every three weeks, was associated with better survival outcomes in pretreated advanced NSCLC whom at least 1 percent tumor cell PDL-1 expression. In Keynote 010 trial compared with chemotherapy OS difference was greater in patients with PDL-1 status >50% who received pembrolizumab, median 8.2 versus 16.9 months [14,15].

Despite clinical benefits, immuno-therapies can cause uniq side effects which is called immune-related adverse events. These side effect include dermatologic, gastro-intestinal, hepatic, endocrine, and other less common inflammatory events. Rarely fulminant and even fatal toxicities may occur with immune checkpoint inhibitors. In general, treatment of moderate or severe irAEs requires interruption of the checkpoint inhibitor and the use of glucocorticoid immunosuppression. Treatment of side effects are based on the

severity of the observed toxicity. Also the toxicity grade is important for the managment of side effects [16].

In the current study, we aimed to present the contribution and reliability of the use of immunotherapy to the survival of NSCLC patients who had received at least one frontline treatment, as a single center experience.

Methods:

Between 2015 and 2021, totally 37 patients with metastatic pretreated NSCLC who have received immunotherapy were included in this study. Patients who could not complete their treatment due to financial and non-illness reasons and those who died for reasons other than cancer, and the patients with ECOG PS 3 and 4 were excluded from data analysis. Patients' data were retrospectively obtained from patients charts with respect to age, number of metastatic sites, treatment choice, duration of treatment, PDL-1 status, survival outcomes and toxicities. The Local Ethics Committee of Istanbul Medipol University approved the study on June 2021 with E-10840098-772-02-2508 decision number.

PDL-1 Expression Assessment: The PDL-1 values of the patients were evaluated with the SP142 method in patients receiving atezolizumab and with the 22C3 method in patients receiving nivolumab.

Previous Treatment: As first-line therapy, 13 of 17 patients with adenocarcinoma histology received a paclitaxel-platinum regimen, while 4 received a pemetrexed-platinum regimen. Of 8 patients with squamous histology, 6 received paclitaxel-platinum and 2 received gemcitabine-platin chemotherapy regimen. Twelve patients using immunotherapy in the third-line received platinum-based doublet chemotherapy in the frontline setting, while they received gemcitabine-docetaxel chemo-therapy regimen in the second-line treatment.

Statistical analysis:

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Survival analysis and curves were established according to the Kaplan-Meier method and compared by the long-rank test. PFS was defined as the time from diagnosis to the last follow-up and the time until relapse as being the time from diagnosis to the first evidence of relapse. In addition, OS was described as the time from diagnosis to the date of the patient's death or last known contact. Univariate and multivariate analysis of prognostic factors related to survival were performed by the Cox proportional hazards model. Multivariate p values were used to characterize the independence of these factors. The 95% confidence interval (CI) was used to quantify the relationship between survival time and each independent factor. All p values were two-sided in tests and p values less than or equal to 0.05 were accepted to be statistically significant.

Results:

Twenty-nine (78.4%) of patients were men and 8 of patients (21.6%) were woman with median age of 61 years (range:42-80). At the initial diagnosis, the majority of patients (64.9%) had advanced stage. Brain metastasis were detected in 15 patients (40.5%) at the initial diagnosis or during treatment. Histopathologically, most patients had adenocarcinoma (n=23, 62.2%). Eight patients had type 2 diabetes mellitus, ten patients had hypertension, in addition seven of patients had chronic obstructive lung disease. Patients and tumor characteristics are shown in Table 1.

PDL-1 positivity in adenocarcinoma histology was 52.2%, response rate to immunotherapy was 91.3%, while PDL-1 positivity in squamous cell histological subtype was 71.4% and response rate to immunotherapy was 85.7%. PDL-1 expression status was classified

Table 1. Patient and tumor characteristics

Characteristics	n	%
Total patients	37	
Age,years		
Median, range	61 (42-80)	
Gender		
Male	8	21.6
Female	29	78.4
Histopathological type		
Adenocarcinoma	23	62.2
Squamous cell carcinoma	13	35.1
Others	1	2.7
Initial clinical TNM stage		
Stage III	13	35.1
Stage IV	24	64.9
ECOG PS		
0	15	40.5
1	8	48.6
2	4	10.8
Tumor PD-L1 expression		
< 1%	21	63.6
1-49 %	8	24.2
>50%	4	12.1
Oncodriver mutation		
Absent	34	91.9
Present	3	8.1
Previous chemotherapy		
1	25	67.6
≥2	12	32.2
Choice of immunotherapy agent		
Nivolumab	15	40.5
Atezolizumab	22	59.5

Table 2: Response rates according to the RECIST 1.1

Response rate	n (%)
Complete response	0
Partial response	13 (35.1)
Stable disease	21 (56.8)
Progressive disease	16 (8.1)
Objective response rate (CR+PR)	13 (35.1)

*CR: Complete response, PR: Partial response,

as <1% in 21 (63.6%), 1-49% in 8 (24.2%) and >50% in 4 (12.1%) patients. There were three patients with presence of driver mutation as EGFR mutation who had adenocarcinoma histology. Therefore, they received targeted therapy in front-line setting. While 25 (67.6%) patients received immunotherapy in the second line setting, 12 patients (32.2%) received in the third and subsequent lines. Atezolizumab was preferred in 22 (59.5%) of these patients and nivolumab in 15 (40.5%) of them. The median cycles and duration of treatment were 5 (range: 2-24) and 3.7 months (range: 1.7-29.6).

Of the 22 patients who were treated with atezolizumab, 5 (20.8%) had partial response (PR) and 14 (58.3) had stable disease (SD). The PDL-1 expression level was measured in 22 of these patients, and the status >1% was measured in 10 of patients (54.2%). Twelve (54.5%) of the 22 patients used atezolizumab as a second line therapy.

Of the 15 patients who received nivolumab, 8 (53.3%) had PR and 7 (46.7%) had SD. The PDL-1 status was measured in 13 (86.7%) of these patients, and the PDL-1 status was >1% in six patients. Thirteen (86.7%) of 15 patients used nivolumab in second line setting.

Objective response rate (ORR) was 35.1% (Table 2). At a median follow up of 22.5 months, median PFS time was 4.7 months, while median OS time was 24.1 months (Figure1, Figure2). Brain metastasis occurred in 15 patients ongoing or pretreatment which were treated with radiotherapy. Cranial metastasis progressed only in 3 patients after radiotherapy. Pseudo-progression was seen in four patients (10.8%), hyper-progression did not occur in any patients.

Univariate analysis for PFS revealed that gender (p=0.03), age (p=0.005), the presence of brain metastasis (p=0.02), PDL-1 status >1% (p=0.035), ECOG PS (p=0.04) and the good response to frontline treatment (p=0.015) were found to be significant prognostic indicators. It also showed that the presence of brain metastasis (p=0.03), PDL-1 status >1% (p=0.027), good response to frontline treatment (p=0.022) and atezo-lizumab preference (p=0.018) were prognostic

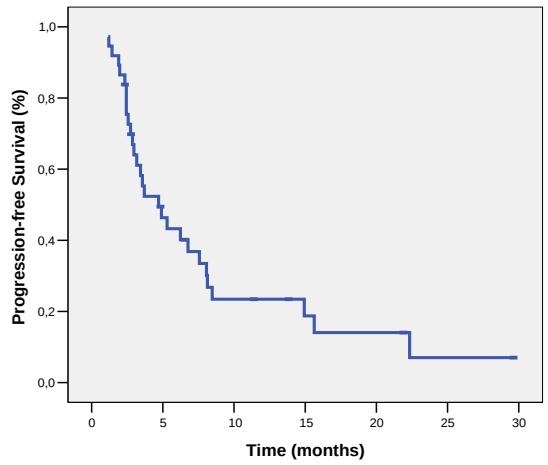

Figure 1: Median progression-free survival curve in patients with metastatic NSCLC

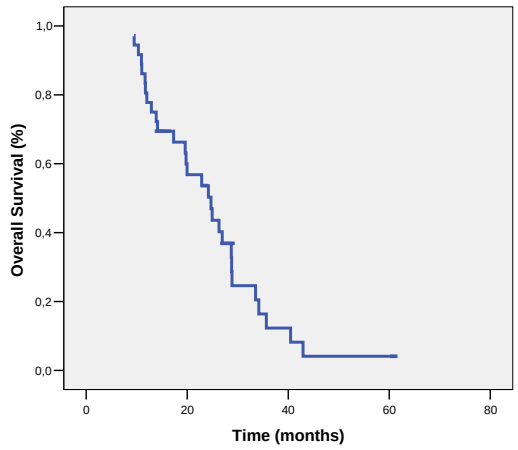

Figure 2: Overall survival curve in patients with metastatic NSCLC

factors for OS (Figure 3, Figure 4). Multivariate analysis indicated that good response to immunotherapy (HR:5.02, p=0.038) and good response to front line treatment (HR: 0.48, p=0.13), atezolizumab preference (HR:3.23, p=0.034) were significantly independent prognostic factors for OS. Figure 5 shows the OS which was significantly better for patients treated with atezolizumab compared with nivolumab arm. Moreover, gender (HR: 5.18, p = 0.0018), age (HR: 0.18, p = 0.003), ECOG PS (HR: 11.3, p =0.002), PDL status >1% (HR:0.32, p= 0.006) and good response to immunotherapy (HR: 0.26, p=0.002) were found to be significant independent prognostic indicators for PFS by multivariate analysis. Table 3 shows multivariate analysis for overall survival and progression-free survival.

The most common grade 3/4 adverse events regarding immunotherapy were pneumonitis in three patients (8.1%), colitis in one patient (2.7%). There was no need to discontinue the treatment due to side effects in neither nivolumab nor atezolizumab. While the dose was delayed in five (33%) of the nivolumab patients due to side effects, the dose was delayed in four (16.7%) of the atezolizumab patients. Moreover, rash (18.2%) and hypothyroidism (24.3%) were common immune-related grade 1-2 adverse events.

Discussion:

Initial treatment approach of advanced NSCLC patients is treating with immunotherapy in combination with platinum-based doublet chemotherapy in front line setting [17]. However, many patients will have treated with only platinum-based doublet chemotherapy. For such patients in the second line setting incorporation of immunotherapy is the preferred approach [3,12,14]. Nivolumab or atezolizumab are appropriate options regardless of tumor PDL-1 expression [4,5]. Pembrolizumab is an option for the tumors with at least >1% of PDL-1 status [6]. There is no data directly comparing these agents, so the choice among immunotherapies differs between centers by local practice and cost-effectiveness.

When the studies were evaluated, the median contribution of immunotherapy to overall survival for nivolumab, atezolizumab and pembrolizumab ranged from 9 to 13.8 months [9,12,15,18,20]. In our study, there were no patients who received pembrolizumab. Unlike, our real life experience with nivolumab

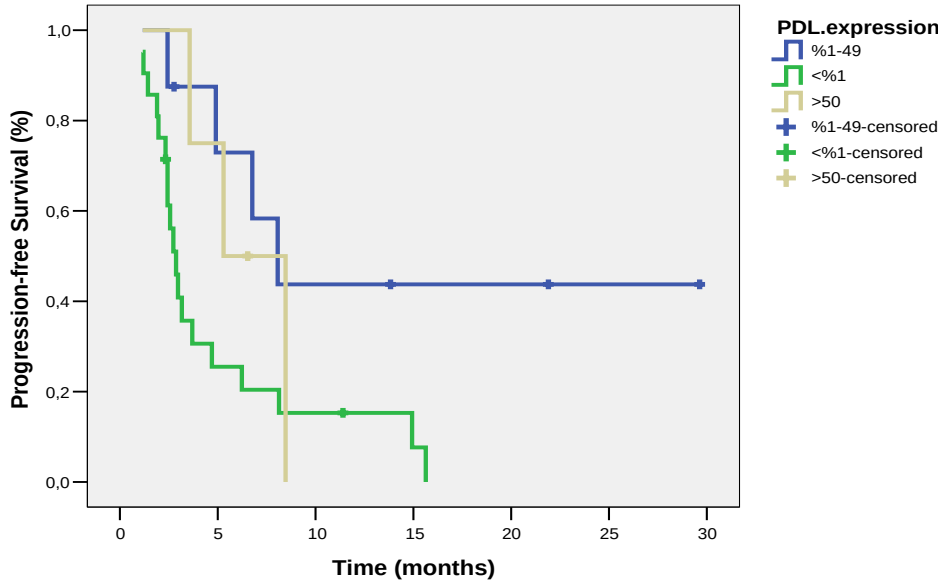

Figure 3: Progression Free Survival curves according to the PD-L1 expression

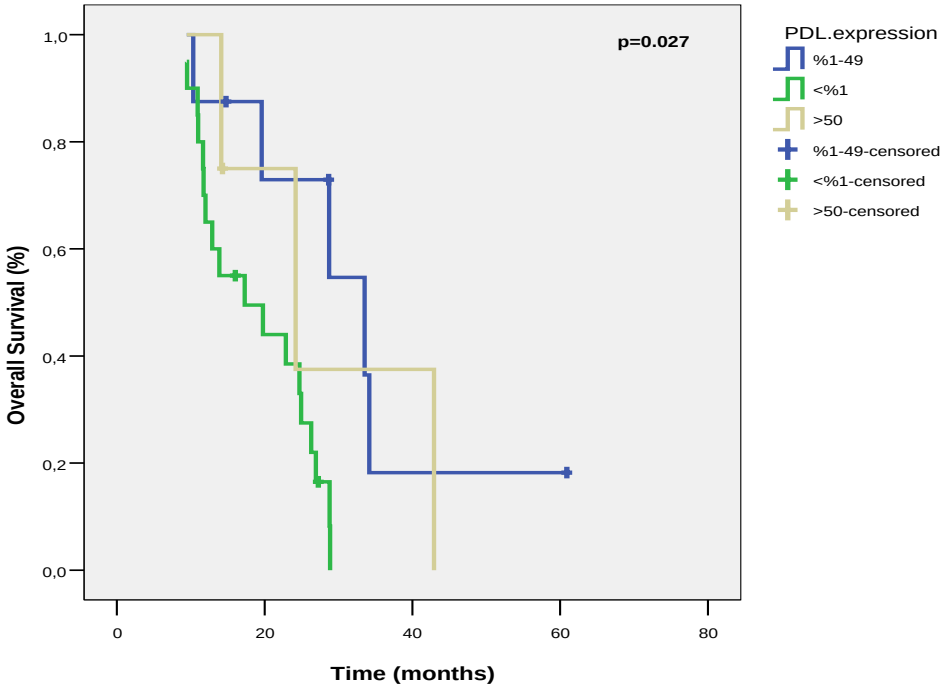

Figure 4: Overall survival curves according to the PD-L1 expression

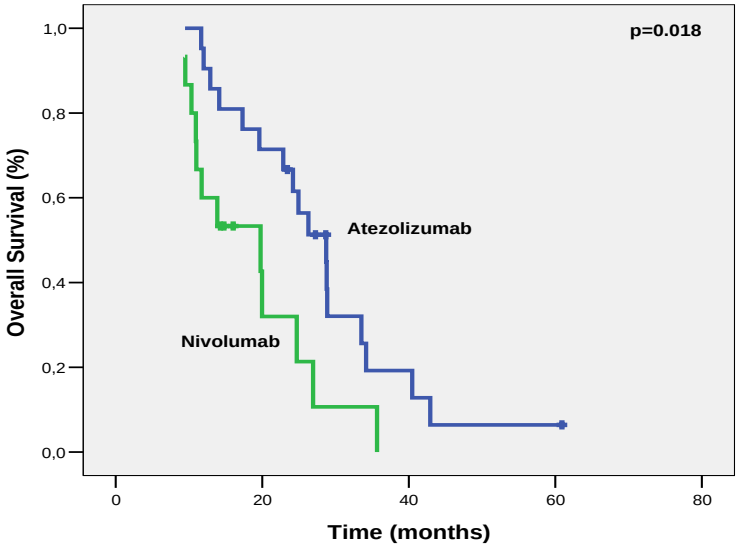

Figure 5: Overall survival curve for patients treated with atezolizumab compared with nivolumab

Table 3: Multivariate analysis for Overall survival and progression-free survival

Factor	X^2	p	HR	95% CI
Overall survival				
Response to immunotherapy	4.29	0.038	5.02	1.09-7.12
Presence of response to first-line chemotherapy	6.23	0.013	0.48	0.27-0.85
Immunotherapy type (Atezo vs Nivo)	4.47	0.034	3.23	1.09-4.09
PD-L1 expression (<1% vs 1-49% vs ≥50%)	3.23	0.072	0.45	0.19-1.07
Presence of brain metastasis	2.94	0.086	0.43	0.16-1.12
Progression-free survival				
Gender	6.98	0.0018	5.18	1.51-7.76
Age (<60 vs ≥60)	6.44	0.003	0.18	0.07-0.72
EGOG PS at the time of immunotherapy (0-1 vs 2)	8.71	0.002	11.3	2.09-19.1
PD-L1 expression (<1% vs 1-49% vs ≥50%)	7.65	0.006	0.32	0.24-0.72
Response to immunotherapy	9.81	0.002	0.26	0.11-0.60
Presence of brain metastasis	0.19	0.65	1.25	0.45-3.47
Presence of response to first-line chemotherapy	0.91	0.33	1.29	0.76-2.17

* HR: hazard ratio, CI: confidence interval, ECOG PS:

and atezolizumab is not similar to literature in terms of OS with median 24.1 months. This situation can be explained by the longer median follow-up period and the small sample size. On the other hand, median PFS interval was 4.7 months as similar to the literature [7,12,14]. One of the reasons for the longer overall survival in our study may be associated with the PDL-1 value >1% in 17 of 39 patients. In previous studies, the ORR with nivolumab was 19% in the squamous histological subtype, 20% in the non-squamous subgroup, while the ORR was 14% with atezolizumab [7,12,14]. However, in our study, ORR was 35.1%. Thus, our findings were not compatible with respect to OS and ORR [7,12,14,19]

In our study we showed that PDL-1 expression might differ according to the histologic type of the lung cancer. While PDL-1 positivity was 71.4% in the squamous cell subgroup, it was 52.2% in the group with adenocarcinoma. Similar studies in the literature determined the PDL-1 positivity in tumor cells was 56.2% in squamous cell carcinoma and 39.9% in adenocarcinoma [17]. One possible explanation for this difference may be that PDL-1 positivity is associated with smoking and squamous cell cancer is more frequently associated with smoking [20]. Previously studies showed the response to immunotherapy was worse in tumors with driver mutation [7,12,14]. In our study only three of patients have had driver mutation. Thus, no comment could be made.

Clinical trials in the second line setting included patients with stable brain metastasis [7,14]. As known brain metastasis is related with poor prognosis and in our cohort the number of patients with brain metastasis both at initial diagnosis and during treatment was 15 (4.5%). Although the survival contribution of immunotherapy is uncertain stereotactic radiosurgery was applied all of patients in our study.

An important point in drug preference is cost effectiveness. Under the conditions of our country, the use of nivolumab 240 mg every two weeks is a more expensive treatment compared to the use of atezolizumab 1200 mg every 3 weeks. Cost effectiveness is one of the reasons why atezolizumab is preferred more frequently in our center. In our center, 2 patients (5.4%) could not continue treatment due to financial reasons.

In fact there are no clear data to predict immunotherapy treatment response; however the factors found to be associated with longer PFS include; ECOG PS, smoking, liver metastases, lactate dehydrogenase (LDH), and neutrophil-to-lymphocyte ratio(NLR), absence of corticosteroid use and age > 50 years in the literature [23,24]. In our study, gender, age, the presence of brain metastasis, PDL-1 status >1%, ECOG PS and the good response to frontline treatment were found as prognostic factors in univariate analysis for PFS. As well multivariate analysis for PFS revealed that gender (HR: 5.18, p=0.0018), age (HR: 0.18, p=0.003), ECOG PS (HR:11.3, p=0.002), PDL-1 status >1% (HR: 0.32, p=0.006) and good response to immunotherapy (HR:0.26, p=0.002) were significant independent prognostic indicators. However, neither in Phase III CheckMate trials nor in OAK trial PDL-1 status was not found to be prognostic and/or predictive factor for the response [21,22]. Our results were thus not compatible with the literature [21,22].

Treatment-related adverse events of grade 3 or 4 were reported in 7% patients with nivolumab in CheckMate 017 and CheckMate 057 trial; 15% of patients who received atezolizumab in OAK trial [9]. Karak FE et al reported that all-grade immune-related adverse events were reported in around 18% of patients, and were mainly grades 2 and 3 [23]. In our series we reported grade 3-4 adverse events in four patients (10.3%) which was the pneumonitis in three patients, colitis in one patient. Any of treatment related

endocrine side effects were seen in eight patients (20.3%).

The small sample size and the retrospective design of our study could be considered as significant limitations and might have influenced these results. On the other hand, the long follow-up period and the management of immune-related side effects according to new guidelines were the positive aspects of our study. Therefore, we believe that our findings contribute to the literature, because we analyzed immunotherapy agents in both second and later lines, and in high PDL-1 positive patients with metastatic NSCLC as a single center experience.

In conclusion, our results indicate that both atezolizumab and nivolumab are active agents with good safety profile in second and later lines treatment for patients with metastatic NSCLC. Our real-life data is compatible with the results of previous clinical trials. However, the fact that the effectiveness is in a more PD-L1 positive group shows the need to identify predictive factors necessary to identify patients who will benefit from these drugs in the future.

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Original Article

A Comparison of the BEAM and BuCyE Conditioning Regimens for Autologous Stem Cell Transplantation in Lymphoma: A Single-Center Experience

Lenfomada Otolog Kök Hücre Transplantasyonu için BEAM ve BuCyE Hazırlama Rejimlerinin Karşılaştırması: Tek Merkez Deneyimi

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ABSTRACT

Introduction: High-dose chemotherapy together with autologous stem cell transplantation (ASCT) is a commonly used treatment modality in patients with relapsed/refractory Hodgkin's lymphoma (HL) or non-Hodgkin's lymphoma (NHL). The aim of this study was to investigate the efficacy and toxicity of BuCyE (busulfan, cyclophosphamide, and etoposide) and BEAM (carmustine, etoposide, cytarabine, and melphalan) conditioning regimens in patients with relapsed/refractory lymphoma scheduled for ASCT.

Methods: Between December 2018 and November 2019, 24 patients with relapsed or refractory HL (n=16) and NHL (n=8) who underwent ASCT following BEAM (n=12) and BuCyE (n=12) preparative regimens were analyzed retrospectively at Bone Marrow Transplantation Unit of Abdurrahman Yurtaslan Ankara Oncology Training and Research. The groups were compared in terms of patient characteristics, hematopoietic engraftment time, toxicity profiles, and progression free survival (PFS).

Results: No significant differences were detected between the groups with regard to age, gender distribution, ecog, sorror score, diagnosis, pre-ASCT stage (early/late), chemotherapy line, pre-ASCT response and pre-ASCT radiotherapy ($p>0.05$). The median number of infused CD34+ cells/kg, neutrophil and platelet engraftment statuses, duration of hospitalization, need for erythrocyte and platelet transfusion of BuCyE and BEAM groups were found to be similar ($p>0.05$). More patients in the BuCyE group developed mucositis and febrile neutropenia, but this difference was not statistically significant ($p>0.05$). At a median follow-up of 13 months(1–21 months) after ASCT, the median PFS could not be reached. No difference was found in PFS between regimes ($p = 0.68$).

Discussion and Conclusion: BuCyE followed by ASCT is an effective conditioning regimen in relapsed/refractory lymphoma patients. This regimen may be an important treatment option as a substitute for carmustine containing regimens. However, in the absence of prospective trials, it is difficult to suggest a conditioning regimen due to the low level of evidence. It is important to participate in ongoing clinical trials.

Keywords: Lymphoma, autologous stem cell transplantation, BuCyE, BEAM

ÖZET

Giriş ve Amaç: Otolog kök hücre transplantasyonu (OKHN) ile birlikte uygulanan yüksek doz kemoterapi, relaps/refrakter Hodgkin lenfoma (HL) veya Hodgkin dışı lenfoma (NHL) olan hastalarda yaygın olarak kullanılan bir tedavi yöntemidir. Bu çalışmanın amacı, OKHN planlanan relaps/refrakter lenfomalı hastalarda BuCyE (busulfan, siklofosfamid ve etoposit) ve BEAM (karmustin, etoposit, sitarabin ve melfalan) hazırlama rejimlerinin etkililiğini ve toksisitesini araştırmaktır.

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Yöntem ve Gereçler: Aralık 2018 ile Kasım 2019 arasında BEAM (n=12) ve BuCyE (n=12) hazırlık rejimleri ile OKHN yapılan nükseden veya dirençli HL (n=16) ve NHL (n=8) olan 24 hasta, Abdurrahman Yurtaslan Ankara Onkoloji Eğitim ve Araştırma Kemik İliği Nakli Ünitesi'nde incelendi. Gruplar hasta özellikleri, hematopoietik engraftman süresi, toksisite profilleri ve progresyonsuz sağkalım (PFS) açısından karşılaştırıldı.
Bulgular: Gruplar arasında yaş, cinsiyet dağılımı, ecog, sorror skoru, tanı, OKHN öncesi evre (erken/geç), kemoterapi sayısı, OKHN öncesi yanıt ve OKHN öncesi radyoterapi açısından anlamlı farklılık saptanmadı (p>0.05). BuCyE ve BEAM gruplarının ortalama infüze edilen CD34+ hücre/kg sayısı, nötrofil ve trombosit engraftman durumları, hastanede kalış süreleri, eritrosit ihtiyacı ve trombosit transfüzyonu benzer bulundu (p>0.05). BuCyE grubunda daha fazla hastada mukozit ve nötropenik ateş gelişti, ancak bu fark istatistiksel olarak anlamlı değildi (p>0.05). OKHN'den sonraki 13 aylık (1–21 ay) medyan takipte, medyan PFS'ye ulaşılamadı. Rejimler arasında PFS'de fark bulunmadı (p=0.68).
Tartışma ve Sonuç: BuCyE'yi takiben OKHN, relaps/refrakter lenfoma hastalarında etkili bir hazırlık rejimidir. Bu rejim, karmustin içeren rejimlerin yerine geçebilecek önemli bir tedavi seçeneği olabilir. Bununla birlikte, ileriye dönük çalışmaların yokluğunda, düşük düzeyde kanıt nedeniyle bir hazırlama rejimi önermek zordur. Devam eden klinik araştırmalara katılmak önemlidir.

Anahtar Kelimeler: Lenfoma, otolog kök hücre nakli, BuCyE, BEAM

Introduction

Most patients with Hodgkin lymphoma (HL) are cured with initial therapy. However, 5-10 % of the patients have a treatment-refractory disease and 10-30% will relapse following standard therapy. Although significant advances have been achieved in the treatment of non-Hodgkin's lymphoma (NHL), 40–60% of the patients still relapse or have a treatment-refractory disease [1].

Many randomized studies have shown significant improvements in progression-free survival (PFS) and event-free survival (EFS) with high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT) in relapsed/refractory HL and NHL [1–3].

The most commonly used high-dose conditioning regimens in relapsed/refractory HL and NHL patients are BEAM (carmustine, etoposide, cytarabine, and melphalan), BEAC (carmustine, etoposide, cytarabine, and cyclophosphamide), CBV (cyclophosphamide, carmustine, etoposide), BuCyE (busulfan, cyclophosphamide, etoposide) and combination regimen with total body irradiation. BEAM is the most commonly preferred HDC regimen among these [4, 5]. The number of randomized studies comparing these regimens to date is quite low. Advances in conditioning regimens and supportive therapy have resulted in a reduction in transplant-related mortality. Although the search for a different regimen continues, recent supply and cost issues for carmustine have created an urgent need for alternative conditioning regimens [6].

The aim of this study was to investigate the efficacy and toxicity of BuCyE and BEAM conditioning regimens in patients with relapsed/refractory HL or NHL scheduled for ASCT.

Materials and methods

In this study, relapsed or refractory NHL or HL patients who received ASCT after salvage chemotherapy at Abdurrahman Yurtaslan Ankara Oncology Education and Research Bone Marrow Transplantation Unit between December 2018 and November 2019 were retrospectively analyzed. The patients with relapsed or refractory NHL and HL who had been diagnosed histopathologically were

Table 1. BuCyE and BEAM chemotherapy regimens

BuCyE protocol		BEAM protocol	
Busulfan (mg/kg)	16 (-7, -6, -5, -4. days)	Carmustine (mg/m^2)	200 (-7. day)
Cyclophosphamide (mg/kg)	120 (-3, -2. days)	Etoposide (mg/m^2)	200 (-6, -5, -4, -3. days)
Etoposide (mg/m^2)	400 (-3, -2. days)	Cytarabine (mg/m^2)	200 (-5, -4, -3, -2. days)
		Melphalan (mg/m^2)	140 (-2. day)

Table 2. Patient characteristics of all patients (n = 24)

Parameters	BEAM (n = 12)	BuCyE (n = 12)	P value
Age (median)	40 (20-59)	36,5 (27-65)	0,51
Gender (M/F)	9/3	10/2	1
ECOG (0/1)	5/7	4/8	1
Sorror Score (0/1-2)	10/2	11/1	0,6
Diagnosis (HL/NHL)	8/4	8/4	1
Disease type			
HL			
NS	5	5	
MC	2	2	
LR	1	-	
LD	-	1	
NHL			
DLBCL	3	3	
BL	1	1	
Pre-ASCT Disease Stage (I-II/ III-IV)	4/8	2/10	0,64
Chemotherapy Line (1-2/ ≥3)	7/5	5/7	0,41
Pre-ASCT Response (CR-PR/Progresyon)	11/1	11/1	1
RT (yes/no)	2/10	2/10	1

M: Male, F: Female, HL: Hodgkin's lymphoma, NS:Nodular Sclerosis , MC: Mixed Cellularity, LR: Lymphocyte Rich, LD: Lymphocyte Depleted, NHL: Non-Hodgkin's lymphoma, DLBCL: Diffuse large B cell lymphoma, BL: Burkitt lymphoma, ASCT: Autologous stem cell transplantation, CR: Complete remission, PR: Partial remission, RT: Radiotherapy.

accepted as suitable candidates for ASCT. All cases enrolled in the study were assessed in terms of chemosensitivity. The other inclusion criteria of the study were age <70 years, adequate heart, lung, liver, and kidney reserves, sufficient hematopoietic function, and Eastern Cooperative Oncology Group performance status of one or zero prior to ASCT. The study involved a total number of 24 patients with lymphoma scheduled for ASCT. Among these patients, 12 cases received BuCyE regimen, while BEAM was applied to 12 patients as preparative regimen prior to ASCT (Table 1). Successful neutrophil engraftment was accepted as an absolute neutrophil count of $\geq 1\times10^9$/L attained for one day, while platelet count $\geq 20\times10^9$/L without a need for platelet transfusion on the first consecutive three days after platelet engraftment was considered to be a successful platelet engraftment procedure. Treatment response was first evaluated one month after ASCT performed, then by 3-months intervals within the first 2 years. The groups were compared in terms of patient characteristics, hematopoietic engraftment time, toxicity profiles, and PFS. PFS was calculated as the time between the day of ASCT and data collection or exitus.

Table 3. Hospitalization process and findings after ASCT

Parameters	BEAM (n =12)	BuCyE (n = 12)	P value
Duration of diagnosis to ASCT (months) (median)	21 (4-214)	27,1 (9-91)	0,41
Diagnosis to transplant > = 24 months HL/NHL	4/1	7/1	
Diagnosis to transplant < 24 months HL/NHL	4/3	1/3	
Duration of Hospitalization (days)	22 (19-26)	22,5 (19-35)	0,29
Infused CD34 kg/cell (median)	9,8 (4,7-14)	6,59 (3,1-16,3)	0,14
Neutrophil engraftment (days) (median)	10(8-10)	10 (9-17)	0,09
HL (median)	10 (8-10)	10 (9-17)	
NHL (median)	10 (8-10)	10 (9-12)	
Platelet engraftment (days) (median)	11 (6-19)	10 (9-32)	0,35
HL (median)	12 (9-19)	10 (9-26)	
NHL (median)	10 (6-12)	12 (9-32)	
Need of ES transfusion(yes/no)	6/6	5/7	0,68
HL patients given ES transfusion	2	5	
NHL patients given ES transfusion	4	-	
Need of PLT transfusion (1-2/ ≥3)	8/4	6/6	0,4
HL patients given PLT transfusion (1-2/ ≥3)	4/4	4/4	
NHL patients given PLT transfusion (1-2/ ≥3)	4/-	2/2	
Mucositis (yes/no)	0/12	3/9	0,22
HL patients with mucositis	-	1	
NHL patients with mucositis	-	2	
Febrile Neutropenia (yes/no)	7/5	8/4	0,67
HL patients with febrile neutropenia	3	7	
NHL patients with febrile neutropenia	4	1	

ASCT: Autologous stem cell transplantation, HL: Hodgkin's lymphoma, NHL: Non-Hodgkin's lymphoma, ES: Erythrocyte suspension, PLT: Platelet.

Statistical analysis

Statistical analyses were performed with IBM SPSS (Version 26) software. Demographical data were summarized with descriptive statistics. Numerical variables were presented as median (minimum-maximum), categorical variables were presented as ratios. To compare groups, Mann Whitney U tests were used for numerical variables and Chi-square test was used for categorical variables. Kaplan-Meier survival analysis performed for PFS and log-rank test was applied to assess survival difference among groups. $P \leq 0.05$ was regarded as statistically significant.

Results

The median age of the patients was 38 (20-65). Of the patients, 33.3% had NHL and 66.7 % had HL. There were 19 (79.2%) male patients and 5 (20.8%) female patients. The median time between diagnosis and ASCT was 21 months (4-214) and 27.1 months (9-91) in BEAM and BuCyE groups, respectively. The characteristics of all patients are included in Table 2.

No significant differences were detected between the groups with regard to age, gender distribution, ecog, sorror score, diagnosis, pre-ASCT stage (early/late), chemotherapy line, pre-ASCT response and pre-ASCT radiotherapy ($p>0.05$) (Table 2). Median number of infused CD34+ cells/kg, neutrophil and platelet engraftment statuses, duration of hospitalization, need for erythrocyte and platelet transfusion of BuCyE and BEAM groups were found to be similar ($p>0.05$). More patients in the BuCyE group developed mucositis and febrile neutropenia, but this difference was not statistically significant ($p>0.05$) (Table 3).

At a median follow-up of 13 months (1–21 months) after ASCT, the median PFS could not be reached, and no difference was determined in PFS between the regimes ($p = 0.68$) (Figure 1).

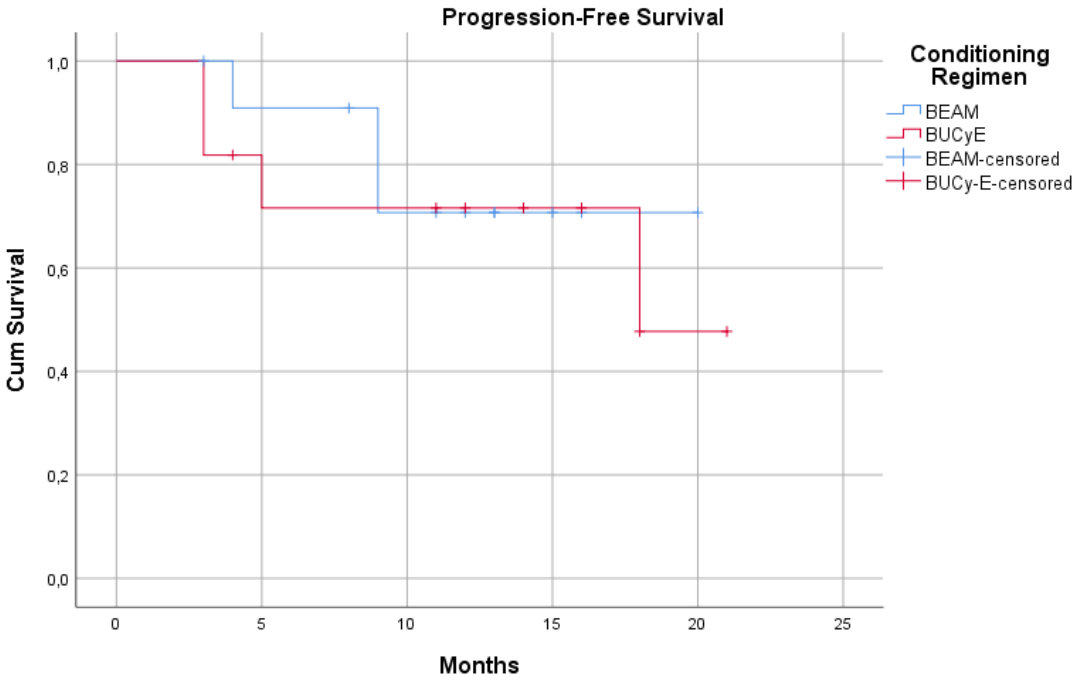

Figure 1. At a median follow-up of 13 months (1–21 months) after ASCT, the median PFS could not be reached and no difference was obtained in PFS between the regimes (p = 0.68).

Discussion

Despite the advances in modern chemotherapy, a significant proportion of patients with NHL or HL either never achieve remission or relapse early. For the vast majority of these patients, HDC followed by ASCT remains the best option for a long-lasting complete response. The most popular conditioning protocol for ASCT in lymphoma is BEAM.

Recent supply and cost issues, the high rate of mucositis requiring parenteral nutrition, and the high incidence of chronic interstitial pulmonary fibrosis for carmustine have created an urgent need for alternative conditioning regimens [6].

The resulting cost of carmustine, both drug-related and of managing toxicities, have spurred the development of novel regimens that replace this agent.

In several studies, bendamustine, thiotepa, fotemustine, lomustine, and mitoxantrone, have been examined as substitutions for carmustine in the BEAM regimen, resulting in similar or superior efficacy with a reduction in toxicity [7-10].

However, the lack of randomized trials using these agents and the fact that they include different study populations with differing proportions of histologies make it difficult to compare across studies.

Hanel M et al. conducted a study on 53 patients with HL or NHL who received high dose BuCyE conditioning regimen and investigated the efficacy and toxicity of BuCyE used as a preparative regimen prior to ASCT. In the evaluation of toxicities, mucositis (79%) and hepatic toxicity (15%) were found to be the most common non-hematological toxicities which were seen in 52 subjects, while three patients (5.8%) experienced severe veno-occlusive disease. In that study, the rate of treatment-related mortality was found as 3.8%. The authors concluded that BuCyE was an effective and well-tolerated conditioning regimen in patients with HL and NHL [5]. In our study,

none of the patients had veno-occlusive disease and in the BuCyE group, the rate of mucositis was 25% (n=3/12) and treatment-related mortality was not.

Singer S. et al. [11] retrospectively compared the BEAM and BuCyE for patients with relapsed NHL undergoing AHCT. After a median follow-up of 3.9 years for BEAM and 4.3 years for BuCyE from AHCT, PFS was found similar between the two conditioning regimens. In this study; it was reported that the number of CD34 infused was higher in the BuCyE group, the platelet engraftment time and hospital duration was shorter than in the BEAM group. In terms of adverse effects, mucositis was significantly more common in the BuCyE group, whereas sinusoidal obstruction syndrome was more common in the BEAM group.

Singer S. et al. [12] retrospectively compared the BEAM and BuCyE for patients with relapsed HL undergoing AHCT. They reported that the use of BEAM conditioning before AHSCT resulted in a statistically significant PFS, OS and lower relapse compared to BuCyE. In this study; it was reported that the number of CD34 infused was higher in the BuCyE group and the platelet engraftment time was shorter than in the BEAM group. They found the length of hospital stay was significantly shorter for the BEAM group and overall toxicities did not differ significantly between the two groups except for high rates of mucositis with BuCyE.

Berber et al. [13] compared 31 patients who received BuCyE and 11 patients who received BEAM in their study. No difference was obtained between the groups as regards the neutrophil and platelet engraftment duration and need for erythrocyte and platelet suspension during the transplantation. Also, mucositis, nausea, vomiting, diarrhea, infectious complications, and transplant-related mortality were found as similar. No statistically significant difference was determined between the groups as regards post-transplantation survival, total survival and EFS rates. As a result, BuCyE and BEAM were found as similar in terms of toxicity profile, and it was maintained that BuCyE could be an alternative preparation regimen. In our study, no difference was determined between both groups in terms of hospitalization duration, neutrophil and platelet engraftment duration, need for erythrocyte and platelet suspension, mucositis and febrile neutropenia, and thus it is similar to the study by Berber et al as regards the results.

In their study, Gunduz et al. [14] reported that in the patients given a BEAM (n=10) and BuCyE (n=10) preparation regimen, neutrophil and platelet engraftment duration, 100^{th} day remission state, hospitalization period, post-transplantation relapse and death, and need for total erythrocyte and platelet suspension were similar in both groups, but survival period is longer in the group receiving BEAM (55.25±15.29 vs. 12.12±4.02 months, $p = 0.02$). In our study, adverse effect profile, support treatment and hospitalization period were similar, and no difference was determined in PFS.

As a result, a small number of patients and a short follow-up time are insufficient to derive firm conclusions. However, a BuCyE conditioning regimen prior to ASCT was a well-tolerated and effective treatment for relapsed/refractory NHL and HL. This regimen may be an important treatment option as a substitute for carmustine containing regimens. Since, carmustine supply and cost issues urge for a search for alternative conditioning regimens.

However, in the absence of prospective trials, it is difficult to suggest a conditioning regimen due to the low level of evidence. It is important to participate in ongoing clinical trials.

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Original Article

Impact of Baseline Neutrophil-to-Lymphocyte Ratio on Outcomes of Glioblastoma Multiforme Patients Treated with Standart Concurrent Chemoradiotherapy

Başlangıç Nötrofil-Lenfosit Oranının Standart Eşzamanlı Kemoradyoterapi ile Tedavi Edilen Glioblastoma Multiforme Hastalarının Sonuçları Üzerine Etkisi

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ABSTRACT

Background: Glioblastoma multiforme (GBM) is the most common and miserable prognosis primary brain tumor in adults. Previously neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation has been demonstrated to have both strong predictive and prognostic value in different cancer types, which has rarely been addressed in GBM patients. The aim of this retrospective cohort study was to evaluatethe prognostic value of pretreatment NLR on survival outcomes of GBM patients who were underwent surgery/biopsy followed by definitive chemo-radiotherapy (CRT) and accessibility of a certain cut-off worth for NLR.

Material and Methods: This study was a hospital-based retrospective observational case-series study. This study was designed to identify 144 GBM patients with full pretreatment and treatment records that underwent surgery/biopsy followed by CRT from January 2007 to December 2011 in our clinics. Age, symptoms, laboratory results and treatment modalities of patients were recorded.

Results: The median follow-up time for the whole population was 15.1 (range 1.8-49.9) months, with 95 patients (84.8%) were death at the time of this analysis. NLR cut-off values of 4.3 (AUC:78.4; 95%CI: 64.8-92) for overall- (OS) and 4.1 (AUC:72.7; 95%CI:61-84.1) for local recurrence-free survival (LRFS) were identified, respectively, by using receiver operating curve analysis. Low NLR was associated with significantly longer median OS (23.2 vs. 12.7 months p=0.001), and LRFS (13.9 vs 9.6 months; p<0.001) as well as longer median both of which retained its independent significant association with survival outcomes in the multivariate analysis (p<0.001 for each).

Conclusion: In conclusion, pre-treatment low-NLR values associate with significantly longer OS and LRFS than those presenting with high-NLR. These findings suggest a novel strong and independent prognostic value for baseline NLR which is cheap, reproducible and easy to measure in routine clinical practice.

Keywords: Neutrophil, Lymphocyte, Glioblastoma Multiforme, survival

ÖZET

Giriş: Glioblastoma multiforme (GBM), yetişkinlerde en sık görülen ve en kötü prognoza sahip primer beyin tümörüdür. Daha önce sistemik inflamasyon belirteci olan nötrofil-lenfosit oranının (NLR), daha nadiren ele alınan GBM hastalarında da olmak üzere farklı kanser türlerinde hem güçlü prediktif hem de prognostik değere sahip olduğu gösterilmiştir. Bu çalışmanın amacı; tedavi öncesi NLR'nun cerrahi / biyopsi sonrası küratif kemoradyoterapi (KRT) alan GBM hastasının sağkalım sonuçları üzerindeki prognostik değerini ve NLR için belirli bir eşik değerin erişilebilirliğini değerlendirmekti.

Materyal-Metod: Bu çalışma, hastane temelli, retrospektif gözlemsel bir vaka serisi çalışmasıydı. Bu çalışma, kliniklerimizde Ocak 2007'den Aralık 2011'e kadar cerrahi/biyopsi ve ardından KRT uygulanan ön tedavi ve tedavi kayıtlarına sahip 144 GBM hastasını belirlemek için tasarlanmıştır. Hastaların yaş, semptom, laboratuvar sonuçları ve tedavi modaliteleri kaydedildi

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Bulgular: Tüm popülasyon için medyan takip süresi 15.1 (1.8-49.9) aydı ve bu analiz sırasında 95 hasta (%84.8) öldü. NLR cut-off değerleri curve analizi kullanılarak sırasıyla Genel sağkalım (OS) için 4.3 (EAA: 78.4; % 95 CI: 64.8-92) ve yerel rekürrenssiz sağkalım (LRFS) için 4,1 (AUC: 72.7; % 95 CI: 61-84.1) olarak tanımlanmıştır. Düşük NLR, istatistiksel olarak anlamlı şekilde daha uzun medyan OS (23.2'ye karşı 12.7 ay p = 0.001) ve LRFS (13.9'a karşı 9.6 ay; p <0.001) ve çok değişkenli analiz (her biri için p <0,001) ile de her iki parametre için daha uzun medyan sağkalım sonuçları ile ilişkilendirildi. Sonuç: Sonuç olarak, tedavi öncesi düşük NRL değerleri, yüksek NLR ile başvuranlara göre önemli ölçüde daha uzun OS ve LRFS ile ilişkilidir. Bu bulgular, başlangıç NLR'si için ucuz, tekrarlanabilir ve rutin klinik uygulamada ölçümü kolay yeni, güçlü ve bağımsız bir prognostik değer önerilebilir.

Anahtar kelimeler: Nötrofil, Lenfosit, Glioblastoma Multiforme, sağkalım

Introduction

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults. According to the Stupp protocol the standard treatment incorporates maximal safe surgical resection, trailed by temozolomide chemotherapy concurrent with and adjuvant to focalized brain irradiation [1, 2]. However, the prognosis of such patients is extremely poor even after this aggressive treatment with a reported median and 2 years survival rates of only 14.6 months and 26.5%, respectively [3]. Therefore, GBM is invariably associated with inevitable recurrences and resultant deaths.

Albeit molecular pathology and genetic investigations on search of novel predictive and prognostic markers are advancing on a daily basis, yet there exists no universally accepted marker excluding the O6-methyl-guanine–DNA methyltransferase (MGMT) [4]. Traditional prognostic factors for GBM include the Karnofsky performance status, age, extent of resection, mental status, symptom duration at diagnosis, neurologic functionality, corticosteroid utilization, Mini-Mental State Examination score (MMSE), and radiotherapy dose [5,6]. Although combinations of these conventional factors effectively discriminate patients into groups with significantly differential outcomes they do not incorporate markers of systemic inflammation which may further be beneficial in prognostic sub-grouping of such patients.

Systemic inflammation has been demonstrated to promote local tumor progression and/or metastases by inducing angiogenesis and DNA damage repair system [7,8]. Accordingly, both the predictive and prognostic value of several biomarkers of systemic inflammation has been investigated in various tumor types [9-11]. High neutrophil-to-lymphocyte ratio (NLR) that is usually reflected by neutrophilia and lymphopenia, is one such biomarker that has been suggested to have a strong predictive and prognostic value in different cancer primaries [12-14]. Therefore, explore novel, convenient, practical and cheap biomarkers is necessary. Consequently, in this retrospective cohort study we planned to investigate the effect pretreatment NLR on survival outcomes of GBM patients who were treated with definitive CRT, and accessibility of a certain cut-off worth for NLR that may be utilized as a clinical indicator of survival outcomes in conjunction with promptly used traditional factors.

Methods and Materials:

We designed this retrospective study to identify 144 GBM patients with full pretreatment and treatment records that underwent surgery/biopsy followed by CRT from January 2007 to December 2011 in our clinics. To be eligible for the study, patients had to meet the following inclusion criteria: age ≥18 years, Karnofsky performance score (KPS) of 70 to 100, an adequate bone morrow reserve, hepatic and renal function. Additionally, patients had to have satisfactory preoperative and postoperative cranial magnetic resonance imaging (MRI) and surgery-CRT interval one month after surgery. Patients with any history of previous chemotherapy and/or cranial irradiation were

excluded. The study protocol was reviewed and approved by our Institutional Ethics Committee before collection of patients' data (project noKA 14/37).

Patient Records and Treatment

One month after surgery underwent a CT simulation for three-dimensional radiotherapy treatment planning (RTP). Gross tumor volume (GTV) was delineated on planning CT or its fusion with diagnostic CT/MRI. The RTP for eligible patients was based on GTV, which was restricted to primary tumors T1 contrast-enhancing tumor at MRI (without edema) on preoperative for patients who underwent biopsy or the surgical tumor bed plus any residual enhancing tumor that is seen on the planning scan in patients who underwent resection. The CTV is not defined. The PTV1 should include the GTV with a margin of 1 cm. However some cases may be used to adapt the PTV1 by excluding sensitive structures, such as the optic chiasm, chiasm, and brain stem. The PTV1 is treated with a dose of 40 Gy in 20 fractions. The PTV2 should include the GTV with a margin of 2 cm, and the PTV2 should be treated with 20 Gy in 10 fractions, for a total cumulative dose of 60 Gy. Concurrent chemotherapy consisted of TMZ at a daily dose of 75 mg/m^2 on 7 days a week from the first until the last day of RT. After a 4-week of break, patients received 4-6 cycles of adjuvant TMZ (150-200 mg/m^2 /d) for 5 days every 28 days. Prophylaxis against Pneumocystis carini with either pentamidine or trimethoprim-sulfamethoxazole was mandatory during concurrent RT and TMZ [2]. The available pre-CRT blood data of each patient was utilized to calculate neutrophil-to-lymphocyte ratio before using steroid.

Statistical Analysis

The primary endpoint was the impact of NLR on overall survival (OS) which was defined as the interval between the first day of CRT and death/last visit. Secondary objective was the identification of a particular cut-off value. For this purpose we used receiver operating characteristic (ROC) curve analysis. Survival curves were estimated according to the Kaplan-Meier method, and log-rank tests were used for univariate statistical comparisons. To evaluate the relationship between different variables and survival, a Cox proportional hazard model was used. All tests were two-tailed. A p-value ≤0.05 was considered significant.

Results

A total of 144 patients were reviewed and 112 patients who met the criteria were included in the study.. Patient characteristics are shown in Table 1. At a median follow-up of 15.1 (range 1.8-49.9) months for the whole study population, 95 patients (84.8%) were died. The median 2-years and 4-years OS rates were 15.1 months, 23.5% and 8.8% respectively.

The search for a special NLR cut-off by utilizing ROC analysis in the whole study population demonstrated the cut-off at 4.3 point (AUC:78.4; 95%CI=64.8-92; Sensitivity: 71.9%; Specificity: 69.6%), which was almost same with the cut-off of 4 and 4.73 previously defined one study and letter by Bambury and Alexious respectively [14,15]. Subsequently separated patients at this cut-off point into two groups: Low-NLR (L-NLR≤4.3) and high-NLR (H-NLR>4.3), the comparative survival analysis exhibited that the patients in L-NLR group had significantly longer OS (23.2 vs. 12.7 months; p=0.001) than their counterparts in H-NLR group. (Table 2, Figure 1). Consequently also detected for a special NLR cut-off value for locally recurrence free survival (LRFS) in our cases demonstrated the cut-off value at 4.1 value (AUC: 72.7 (95%CI: 61-84.1), and divided patients cut-off degree into two groups: Low-NLR (L-NLR≤4.1) and high-NLR (H-NLR>4.1). According to comparative LRFS analysis demonstrated that the patient in L-NLR group had paramount extended LRFS (13.9 vs. 9.6 months; p<0.001) than other group in H-NLR (Table 2, Figure 2).

We investigated the potential association between several prognostic factors. A univariate analysis was performed on the following factors: <50 age, sex,≥80 KPS, RTOG RPA classification, ≥3 months symptom duration, type of surgery performed

Table 1. Baseline patients and disease characteristics

Characteristic	N (%)
Age, y	
Median (range)	58 (32-69)
≤50	42 (37.5)
>50	70 (62.5)
Sex	
Male	76 (67.8)
Female	36 (32.2)
KPS	
70-80	39 (34.8)
90-100	73 (65.2)
RTOG RPA Class	
III	43(38.4)
IV	45 (40.2)
V	24 (21.4)
Extent of Surgery	
Complete resection	37 (41.9)
Partial resection	65 (58.0)
Biopsy	10 (0.10)
Symptom Duration	
<3 months	67 (59.8)
≥3 months	45 (40.2)
NLR for OS	
>4.3	46 (41.1)
≤4.3	66 (58.9)
NLR for LRFS	
>4.1	41 (36.6)
≤4.1	71 (63.4)

RTOG Radiation Therapy Oncology Group; KPS, Karnofsky Performance Score; RPA, recursive partitioning analysis; OS, overall survival; LRFS, locally recurrence free survival; NLR, neutrophil lymphocyte ratio

Table 2: Outcomes of overall and local recurrence free survival according to neutrophil-to- lymphocyte ratio

Survival	All patients	NLR ≤cut-off value*; #	NLR >cut-off value*; #	P-value
Overall survival*				
Median (mo, %95 CI)	15.1(12.9-17.3)	23.2(19.5-26.9)	12.7(9.7-15.7)	0.001
2 year (%)	23.5	43.2	13.9	
4 year (%)	8.8	20.6	0	
LRFS#				
Median (mo, %95 CI)	10.8 (9.3-12.3)	13.9 (11.2-16.6)	9.6 (8.3-10.9)	<0.001
2 year (%)	12.9	24.4	9.0	
4 year (%)	6.3	24.4	0	

NLR, neutrophil lymphocyte ratio; LRFS, Locally recurrence free survival * NLR ROC defined cut-off for overall survival ≤4.3, >4.3; #≤4.1, >4.1; NLR ROC defined cut-off for LRFS; mo, months; %95 CI, 95% confidence interval

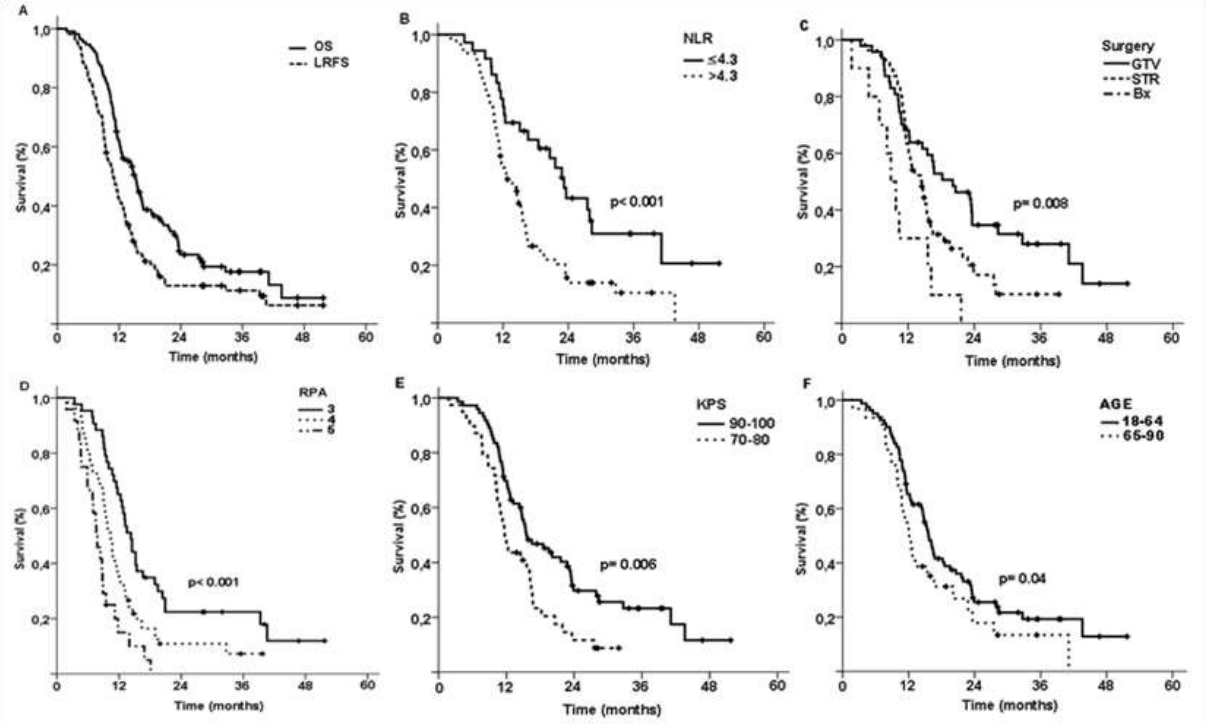

Figure 1:The whole population and comparative subgroup analysis for overall survival and locally recurrence free survival, A: Overall survival and locally recurrence free survival for whole study cohort, B. Neutrophil-to-lymphocyte ratio groups, C. Surgery type, D. Recursive partitioning analysis groups, E. Karnofsky performance score status, F. Age groups

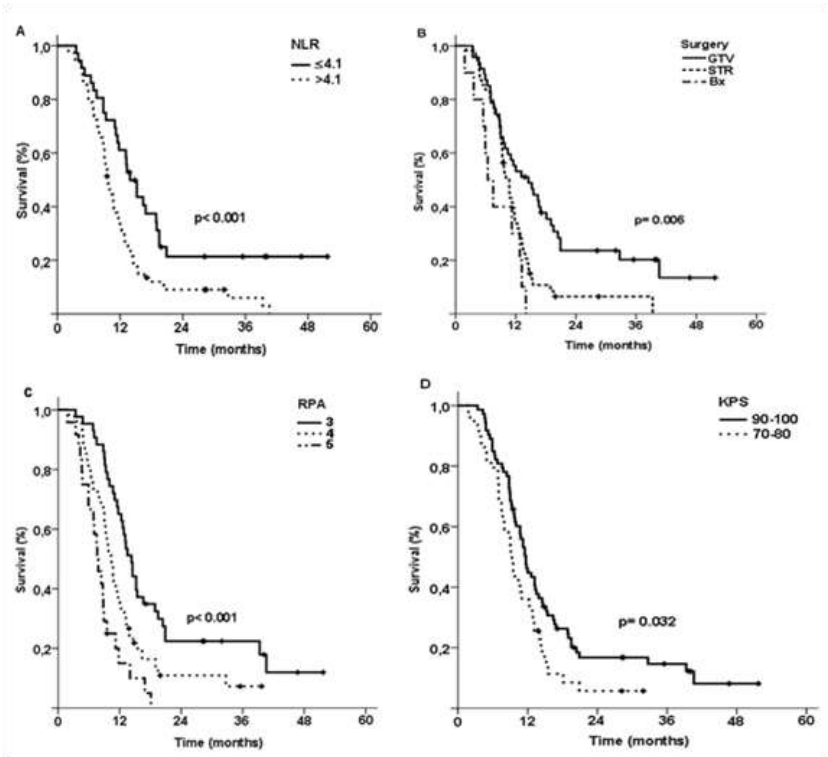

Figure 2: Outcomes of locally recurrence free survival analysis, A. Neutrophil-to-lymphocyte ratio groups, B. Surgery type, C. Recursive partitioning analysis groups, D. Karnofsky performance score status

Table 3: Results of uni and multi-variate analyses for overall survival and local recurrence free survival

Factor	OS Months (%95 CI)	Univariate P-value	Multivariate P-value	LRFS Months (%95 CI)	Univariate P-value	Multivariate P-value
Age, y						
≤50	15.6 (14.0-17.2)	0.03	0.04	11.7 (10.1-13.3)	0.249	
>50	12.1 (10.2-14.0)			9.7 (8.2-11.2)		
Sex						
Male	14.3 (11.1-17.5)	0.29		11.0 (9.8-12.2)	0.138	
Female	16.2 (11.6-20.8)			11.5 (8.0-15.0)		
KPS						
70-80	11.7 (10.1-13.3)	0.007	0.006	9.3 (8.3-10.3)	0.036	0.032
90-100	15.5 (13.0-18.0)			11.7 (10.5-12.9)		
RPA Class						
III	20.6 (12.3-28.9)	<0.001	<0.001	14.3 (12.5-16.1)	<0.001	<0.001
IV	14.5 (10.8-18.2)			10.4 (8.8-12.0)		
V	10.4 (9.2-11.6)			7.6 (5.9-9.3)		
Extent of Surgery						
Complete resection	20.1 (11.5-28.7)			14.5 (9.3-19.7)		
Partial resection	14.5 (12.0-17.0)	<0.001	0.008	10.7 (9.4-12.0)	0.007	0.006
Biopsy	8.9 (6.4-11.4)			6.4 (3.9-8.9)		
Symptom Duration						
<3 months	16.1 (14.5-17.7)	0.041	0.08	12.1 (10.5-13.7)	0.055	
≥3 months	12.1 (10.4-13.8)			9.6 (8.7-10.5)		
NLR						
≤*, #	23.2 (19.5-26.9)	<0.001	<0.001	13.9 (11.2-16.6)	<0.001	<0.001
>*, #	12.7 (9.7-15.7)			9.6 (8.3-10.9)		

RTOG Radiation Therapy Oncology Group; RPA, recursive partitioning analysis; KPS, Karnofsky Performance Score; NLR, neutrophil lymphocyte ratio; %95 CI, 95% confidence interval; * NLR ROC defined cut-off for overall survival ≤4.3, >4.3; #≤4.1, >4.1; NLR ROC defined cut-off for LRFS

(gross total resection) were significantly longer OS (p<0.05 for each), excluding the sex (p=0.29) (Table 3).

In multivariate analyses restricted to NLR (L-NLR vs. H-NLR), <50 age, ≥80 KPS, RTOG RPA classification, type of surgery performed (gross total resection), was the variable that retained its independent significance on association with OS time (p=<0.001, 0.04, 0.006, <0.001, 0.008 respectively), except for symptom duration (p=0.08) (Figure 1, Table 3).

We also investigated the potential association between aforementioned prognostic factors and LRFS. The univariate analysis was performed on the following factors: ≥80 KPS, RTOG RPA classification, type of surgery performed (gross total resection) were significantly longer LRFS (p=0.036, <0.001, 0.007 respectively), while other factors could not demonstrate any significance (p>0.05) (Table 3).

In addition multivariate analyses restricted to NLR (L-NLR vs. H-NLR), ≥80 KPS, RTOG RPA classification, type of surgery performed (gross total resection), was the variable that retained its independent significance on association with LRFS time (p=<0.001, 0.032, <0.001, 0.006 respectively) (Figure 1, Table 3).

Discussion

The results of present retrospective investigation suggested a prognostic value for pre-treatment NLR by demonstrating a strong association between the lower NLR ratio and superior LRFS (13.9 vs. 9.6 months; p<0.001) and OS durations (23.2 vs. 12.7 months; p=0.001)in newly diagnosed GBM patients who underwent surgery/biopsy followed by Stupp protocol, which may be used potential prognostic stratification in clinical. Given its relative cost-effectiveness in routine and cheaply be measured in any ordinary oncologic laboratory use, NLR is therefore a

suitable adjunct to other determinants of GBM prognosis.

Even if an important local control due to more than 85% of GBMs, still recurrence within the treatment field. GBMs are characterized by uncontrolled proliferation, diffuse infiltration of adjacent tissues, and revealed to identify prognostic factors in GBM patients enrolled in various clinical trials [17,18]. In the recently years, NLR is novel prognosticator marker allow the identification of inflammation and carcinogenesis which reflect disease biology, and numerous studies have suggested that an increased NLR is collaborated with poor survival of subject with various cancers. But there area limited number of studies in the literatureon this subject in GBM patients [15, 16,19].

In our study demonstrated that high neutrophil infiltration the progression of earlier can become. According to LRFS analysis demonstrated that the patient in H-NLR (NLR>4.1) group had paramount inferior LRFS than other group in L-NLR (NLR≤4.1) (9.6 vs 13.9 months; p<0.001). Although the exact mechanisms behind the role of increase NLR (elevated neutrophils count is associated by a decrease lymphocytes) in cancer worse prognosis effect is not to be explained with the design of our study, its reasonable to anticipate one of the possible mechanisms is association of H-NLR with inflammation, which is neutrophilia have been primary source of circulating VEGF, which has been shown to have a crucial role tumour- related angiogenesis and thus has a near relationship with vascular invasion and metastasis [20], and an inflammatory response inhibits the immune system by depressing the cytolytic activity of immune cell, and secrete tumor growth promoting factor [21-27]. On the other hands, lymphopenia is dependent to the immune escape of tumour cells from tumour-infiltrating lymphocytes (TIL) [28,29], and thus both increase infiltration of tumors and systemic is lymphocytes associated with better response to cytotoxic treatment and prognosis in cancer [30,31]. Therefore elevated neutrophils count is associated by a decrease lymphocytes means that may be immune deficiency of the patient. For this reason, due to with patient H-NRL is not sufficiency immune response, tumor may be progression earlier. Our study demonstrated that the patient in H-NLR group had paramount inferior LRFS than other group in L-NLR. For these reasons in our study thought high neutrophil infiltration that the progression of earlier can become.

Another important results of our study that patients with L-NLR (NLR≤4.3) had significantly longer median (23.2 vs 12.7; p=0.001), 2-year (43.2% vs. 13.9%) and 4-years (20.6% vs 0%) OS rates compared to those with H-NLR (NLR>4.3), suggesting a strong prognostic worth for pre-treatment NLR. In accordance with the first study performed by Bamburay et al. suggested that evaluable NLR>4 presented a worse independent prognostic factor in 84 GBM patients [15]. But Bamburay et al. study had been only 27% patient performed gross totally resection, 24% patients ECOG 2 and 58% patients were able to delivered concurrent chemoradiotherapy plus ≥2 cycles consol-idation TMZ. At the same time author only analyzed overall survival. Different in our cohort, 41.9% patients perform gross totally resection, all of them patients KPS ≥80 and were able to delivered concurrent chemo-radiotherapy plus ≥2 cycles consoli-dation TMZ. The other letter study show that pre-treatment NLR in 51 GBM patients with longer OS (NLR ratio <4.73; p=0.01). In multivariate analyses NLR ratio and extent tumor resection were recognized independent prognostic factor (p=0.01, p=0.025 respect-ively) [16]. Alike ours, these results suggested that L-NLR patients had higher local control than H-NLR therefore reflected longer survival in our study L-NLR patients, or body defenses system and immunity stronger L-NLR patients than H-NLR patients.

In GBM literature conventional prognostic factors were analyzed including age, duration time of diagnosis and surgery, sex, KPS, RTOG RPA classification, type extent of surgery resection. According to uni- and

multivariate analysis except for the sex and symptom duration; the other factors namely ≤50, KPS>80, RTOG RPA class III, gross total resection detected OS (p=0.03, 0.007, <0.001, 0.041 and p= 0.004, 0.006, <0.001, 0.008 respectively) longer than respective counterparts. However, uni- and multivariate analysis for LRFS detected KPS>80, RTOG RPA class III, gross total resection significant longer than respective counterparts (p=0.036, <0.001, 0.007 respectively), other prognostic factor was not significant (p>0.05). During the analysis MGMT was not routinely used by our pathology, therefore we didn't analyze the potential effects of the molecular marker MGMT and NLR value correlation.

Our present study has considerable limitations. First, as with any retrospective single-institution study, unpredictable biases may have influenced our results. Second, we did not have data on MGMT methylation status in our study population. But Han et al. study demonstrated NLR levels did not correlate with O-methylguanine-DNA methyltransferase (MGMT) promoter methylation status, they suggesting that these two prognostic factors may influence clinical outcome via different pathways and mechanisms [32]. Third, our sample size probably small, and we did not analyze possible predictive influence of NLR in GBM patients. Fourth; we did not investigate other potential prognostic factors ie: CPR, platelet to lymphocyte ratio, VEGF, MVP, MMP. Finally, our study warrants further confirmation in large prospective sample cohort studies with a definitive NLR cutoff value.

Conclusions

In conclusion, our study demonstrated that GBM patients presenting pre-treatment L-NLR associated with better immunity status, better response to cytotoxic treatment, prognosis, and so have significantly increased median, long-term survival rates and LRFS than those presenting with H-NLR. Such patients may be beneficial for the selection of individuals requiring more intense treatment, and may lead to a review of our current approaches for treating GBM patients with H-NLR. These findings suggest a novel, strong and independent prognosticator value for baseline NLR, which can easily, routine and cheaply measurable in any ordinary oncologic laboratory.

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Original Article

Salvage Treatment Options for Glioblastoma: Is Re-Operation Beneficial in Early Recurrence?

Glioblastomda Kurtarma Tedavi Seçenekleri: Erken Nükslerde Re-Operasyon Katkı Sağlar mı?

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ABSTRACT

Introduction: We aimed to investigate salvage treatment options for glioblastoma and to explore the role of surgery in early progression.

Methods: The study was designed as a retrospective review of 73 recurrent glioblastoma patients treated between July 2011 and March 2016. Patients were divided into two groups according to time of progression and re-treatments were analyzed for each. Early and late progressions were defined as progression before and after completion of the standard treatment package (≤9 months versus >9 months). Survival analysis were made with Kaplan-Meier method. Survival time comparisons between groups were made with Log-Rank test. Effect of variables on survival times were evaluated with Cox-Regression Analysis.

Results: Median overall survival time from the first diagnosis was 20 months (95% CI 17.0-22.9) and 2-year survival rate was 32.9%. Median time to progression was 10 (1-42) months. Median post progression survival (PPS) time was 8 months (95% CI 6.2-9.8). In multivariable analysis, we found early progression (9 months or less, $p<0.001$) and the use of supportive care after progression ($p<0.001$) as negative prognostic factors for PPS. In late progression, re-operation provided higher rates of PPS than systemic therapy (median 27 vs 10 months, p: 0.005) and supportive care (median 27 vs 3 months, $p<0.001$). However, no significant difference was found between reoperation and supportive care in case of early progression (median 3 vs 1 months, $p=0.143$).

Discussion and Conclusion: Progression is inevitable after standard treatment of glioblastoma. Survival after relapse is considered to be shorter than a year and appropriate patient selection is crucial when deciding on re-treatments. Survival rates of patients with progression earlier than 9 months are lower, and reoperation may not be an ideal option for this group.

Keywords: glioblastoma, reoperation, re-irradiation, temozolomide, bevacizumab

ÖZET

Giriş ve Amaç: Glioblastom, kötü seyirli, erişkinlerde en sık görülen primer beyin tümörüdür. Standart tedavi sonrası hastaların hemen hepsinde progresyon gelişmektedir. Progresyonda re-operasyon, sistemik tedaviler ve re-irradyasyon uygulanabilmektedir. Erken progrese olanların prognozu geç progrese olanlara göre daha kötüdür. Bu çalışmada progresyon zamanına göre kurtarma tedavi seçeneklerinin irdelenmesi amaçlandı.

Yöntem ve Gereçler: Temmuz 2011-Mart 2016 arasında tedavi edilmiş 73 nüks glioblastom tanılı hasta restospektif olarak değerlendirildi. Standart tedavi programı tamamlanmadan önce (≤9 ay) progrese olanlar 'ERKEN', tamamlandıktan sonra (>9 ay) progrese olanlar 'GEÇ' progresyon olarak tanımlandı.

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Her iki grup için kurtarma tedaviler irdelendi. Sağkalım analizleri için Kaplan-Meier metodu kullanıldı. Tek değişkenli analizlerde log rank, çok değişkenli analizde cox-regresyon testi kullanıldı.
Bulgular: İlk tanıdan itibaren genel sağkalım 20 ay (%95 CI 17.0-22.9), 2 yıllık genel sağkalım %32.9 olarak bulundu. Medyan progresyon zamanı 10 (1-42) aydı. Progresyon sonrası genel sağkalım 8 ay (%95 CI 6.2-9.8) olarak saptandı. Çok değişkenli analizde, 9 aydan erken progresyon ($p<0.001$) ve destek tedavi ($p<0.001$) sağkalımı negatif yönde etkileyen faktörler olarak bulundu. Geç progresyonda cerrahinin, sistemik tedaviden (medyan 27'ye karşı 10 ay, p: 0.005) ve destek tedavisinden (medyan 27'ye karşı 3 ay, $p<0.001$) daha iyi sağkalım sağladığı gözlendi. Erken progresyonda ise re-operasyon ve destek tedavisi arasında fark saptanmadı (medyan 3'e karşı 1 ay, p: 0.143).
Tartışma ve Sonuç: Glioblastomda standart tedaviler sonrası progresyon kaçınılmazdır. Progresyon sonrası sağkalım 1 yıldan kısadır ve kurtarma tedavi seçenekleri için uygun hasta seçimi önemlidir. Erken progresyon gösteren hastaların sağkalımı düşüktür ve re-operasyon bu hastalar için uygun olmayabilir.

Anahtar Kelimeler: glioblastoma, re-operasyon, re-irradyasyon, temozolomid, bevasizumab

Introduction

Glioblastoma, also known as Glioblastoma multiforme (GBM) is the most common central nervous system tumor in adults. At present, the standard treatment of GBM is maximal safe resection followed by 60 Gy conventional radiotherapy concurrently with temozolomide (TMZ) and adjuvant maintenance TMZ. Despite the current protocol, most patients progress within a year and have a median survival of 14.6 months [1].

After progression, current treatment options are limited and often ineffective. Surgery is always a tried-out option in selected patients if the tumor is well-suited for surgery [2]. Numerous studies published assessing the role of chemotherapeutic agents and their combinations such as nitrosoureas, TMZ, bevacizumab (BVC), immunotherapeutics and targeted therapies [3,4]. Re-irradiation is also a safe option with the advances of technology. Particularly, fractionated stereotactic radiotherapy has been shown to be useful in progression [5]. However, improvements on survival are insufficient and standard therapy currently is lacking.

Various studies support the conclusion that progressive glioblastoma (pGBM) treatment should be determined on a patient-to-patient basis and careful consideration of factors such as clinical/performance status, age, and quality of life is vital to deciding on treatment [6]. The most discussed topic of pGBM management is the usefulness of re-operation. Concerns about the additional morbidities of surgery, and short survival rates of the disease complicate the decision. Recently, a meta-analysis highlighted the timing of re-operation and showed that early re-intervention is associated with a higher risk of death than late re-intervention [7]. Patients with early progression may have an aggressive tumor moleculer profile, and this may be more important than the therapy itself. The progression time can have a significant impact on the treatment decision of pGBM.

The aim of the study is to demonstrate the prognostic role of the progression time and to investigate time-dependent salvage treatment options for pGBM.

Materials and Methods

Study Design

We retrospectively reviewed the medical records of patients treated for glioblastoma between July 2011 and March 2016. Inclusion criteria were as follows: (1) Patient had to have a radiologically proven progression after 1st treatment according to Response Assessment in Neuro-Oncology (RANO) Criteria [8], (2) patient had to attend follow-

up. Patients who could not be operated and only biopsied were excluded. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study protocol was approved by The Ethics Committee of Cerrahpaşa Medical Faculty (21.02.2020/30332) and all patients had written informed consent.

Initial treatment

All patients underwent maximal debulking surgery, followed by radiotherapy plus TMZ. Total resection was achieved on 35 (48%) patients, while 38 (52%) patients had subtotal resection. Radiotherapy (RT) was administered 60 Gy with conventional fractionation in 6 weeks. RT planning was done in a single-phase treatment plan with a 2-2.5 cm Clinical Target Volume (CTV) and 5mm Planning Target Volume (PTV) margin, including peritumoral edema. TMZ 75 mg/m^2 oral capsule was taken every day during RT. Eight patients (11%) received hypo-fractionated radiotherapy (40 Gy in 3 weeks) due to lower performance status and/or older age. Of these, four patients were administered with concomitant TMZ. After radiochemo-therapy, 6 cycles of adjuvant TMZ (150- 200 mg/m^2) were administered over a 28-day cycle for 5 days (1). Adjuvant TMZ was given 12 cycles in 2 (2.73%) patients and lower than 6 cycles (median 3) in 31 (42.5%) patients. It was stopped earlier due to hematological toxicity in four patients and rapid progression in 27 patients. After completion of treatment, the patients were followed up with a clinical examination and magnetic resonance imaging (MRI) every 2 months. Tumor progression was defined as the evidence of a new contrast-enhancing lesion or a ≥25% increase in size of a known contrast-enhancing lesion or remarkable increase T2/FLAIR abnormality on MRI.

Treatment after progression

Patients eligible for surgery after recurrence were operated as a first option, others were considered for chemotherapy firstly. TMZ was used as in the initial treatment dose, 150-200 mg/m^2, 5 days in every 4 weeks until progression. BVC was administered 10 mg/m^2 every two weeks. Reirradiation was used only in two patients with a hypofractionated scheme (6x5 Gy). Twenty patients were not eligible for any treatment and received only supportive care.

Data Collection

Data collection form includes age (at diagnosis), gender, tumor location, extent of resection, Karnofsky performance score (KPS), initial treatments, date of progressions, salvage treatments, date of death or last follow-up. The progression time was defined as the time from the first surgery to the first evidence of progression. Overall survival (OS) was defined as the time from the surgery to the last follow up or patient's death. Post progression survival (PPS) was defined as the time from the first evidence of progression to the last follow up or patient's death.

Statistical Analysis

All analyses were performed on SPSS package (SPSS 22.0 for Windows; SPSS Inc, Chicago, IL). Imbalances in categorical variables were tested using the Chi-square test. Survival analysis were made with Kaplan-Meier method. Differences were compared with Log-Rank test. Pairwise comparisons were made with Bonferroni correction method. Effect of variables on survival times evaluated with Cox-Regression Analysis with Backward Conditional method. $p<0.05$ values were accepted as statistically significant.

Results

A total of 147 patients were screened. We enrolled 73 eligible cases into our study,

Table 1. Patient and tumour characteristics

	Early progression, ≤9 month, n (%)	Late progression, >9 month, n (%)	All n (%)	p
Age				
<60	20 (64.5)	29 (69.0)	49 (67.1)	0.684
≥60	11 (35.5)	13 (31.0)	24 (32.9)	
Sex				
Male	15 (48.4)	24 (57.1)	39 (53.4)	0.459
Female	16 (51.6)	18 (42.9)	34 (46.6)	
Initial KPS				
≤70	12 (38.7)	9 (21.4)	21 (28.8)	0.107
>70	19 (61.3)	33 (78.6)	52 (71.2)	
Main Location				
Frontal	11 (35.5)	18 (42.9)	29 (39.7)	
Temporal	8 (25.8)	11 (26.2)	19 (26.0)	0.828
Parietal	8 (25.8)	10 (23.8)	18 (24.7)	
Occipital	4 (12.9)	3 (7.1)	7 (9.6)	
Extensiveness				
Single lobe	21 (67.7)	31 (73.8)	52 (71.2)	0.571
Multiple lobes	10 (32.3)	11 (26.2)	21 (28.8)	
First surgery type				
Total	13 (41.9)	22 (52.4)	35 (47.9)	0.377
Subtotal	18 (58.1)	20 (47.6)	38 (52.1)	
Treatment after progression				
Surgery	8 (25.8)	7 (16.6)	15 (20.6)	
Temozolomide	6 (19.4)	19 (45.2)	25 (34.2)	0.227
Bevacizumab	6 (19.4)	7 (16.7)	13 (17.8)	
Supportive care	11 (35.5)	9 (21.4)	20 (27.4)	

median age was 53 (24-79) years. Median overall survival time from the first diagnosis was 20 months (95% CI 17.0-22.9) and 2-year survival rate was 32.9%. Median time to progression was 10 (1-42) months. Median survival time after progression was 8 months (95% CI 6.2-9.8), 6- months and 1-year PPS rate was 58.9% and 30.1%, respectively. The patients were dichotomized into two groups according to the time of progression. Early progression, which was defined progression before completion of the standard treatment package (≤9 months), was seen in 31 patients. Late progression, which was defined progression after completion of the standard treatment package (>9 months), was detected in 42 patients. Two patients were still alive at the time of our review. Isocitrate dehydrogenase (IDH) mutations were positive in five patients, negative in 30 patients and missing in 38 patients. Five of the seven samples examined had methyl guanine methyl transferase (MGMT) promoter-methylation. MGMT status was missing in 66 patients. Demographic characteristics were given in Table 1.

After first progression, the most common treatment was TMZ in 25 (34%) patients. The second and third progressions were demonstrated radiologically with only 16 and 5 patients, respectively. Others experienced rapid clinical deterioration without an MRI diagnosis and did not receive tertiary treatments. (Table 2).

In univariable analysis, male patients had significantly higher PPS times than females (p=0.030). Patients with a score equal to or less than 70 KPS had a shorter PPS time than patients with a score higher than 70 KPS (p=0.088), and patients who had progression after 9 months had higher PPS times than those who did not (p<0.001). When we evaluated the type of treatment after relapse, the supportive care arm had a significantly shorter PPS than the others (p<0.001) (Table 3).

Table 2. Treatment methods after relapse

	First relapse (n=73)	Second relapse (n=16)	Third relapse (n=5)
Temozolomide	25 (34%)	2 (12%)	
Supportive care	20 (27%)	2 (12%)	1 (20%)
Surgery	15 (21%)	3 (19%)	
Only Surgery	8 (11%)		
Surgery + TMZ	3 (4%)		
Surgery + BVC	2 (3%)		
Surgery + RT + TMZ	2 (3%)		
Bevacizumab	13 (18%)		
BVC	11 (15%)	2 (12%)	2 (40%)
BVC + IRI	2 (3%)	6 (38%)	1 (20%)
PCV		1 (6%)	1 (20%)

TMZ:Temozolomide, BVC: Bevacizumab, RT: Radiotherapy, IRI:Irinotecan, PCV: Procarbazine, lomustine, vincristine.

Table 3. Survival rates after progression

	n	Median Survival (Month)	95% Confidence Interval		1- year Survival Rate (%)	p
			Lower	Upper		
Overall Survival after progression	73	8	6.2	9.8	30.1± 5.4	N/A
Age						
<60	49	9	7.0	10.1	32.7 ±6.7	0.642
≥60	24	6	1.2	10.8	25.0 ±8.8	
Gender						
Male	39	10	6.7	13.0	38.5 ± 7.8	0.030
Female	34	6	3.1	8.9	20.6 ± 6.9	
Initial Karnofsky Performance Status						
≤ 70	21	7	2.5	11.5	19 ± 8.6	0.088
> 70	52	9	5.9	12.1	34.6 ± 6.6	
Main Location						
Frontal	28	9	6.9	11.0	32.1 ± 8.8	
Temporal	20	7	6.0	8.0	26.3 ± 10.1	0.516
Parietal	18	9	3.7	14.3	35.3 ± 11.6	
Occipital	7	3	0.4	5.6	14.3 ± 13.2	
Extensiveness						
Single lobe	52	8	5.2	10.8	30.8 ± 6.4	0.658
Multipl lobes	21	9	5.7	12.3	28.6 ± 9.9	
First Surgery						
Total resection	35	9	6.7	11.3	31.4 ± 7.8	0.218
Subtotal resection	38	7	3.6	10.4	28.9 ± 7.4	
Progression time						
≤9 month	31	5	1.9	8.1	9.7 ± 5.3	<0.001
>9 month	42	10	6.0	14.0	45.2 ± 7.7	
Treatment After Relapse						
Temozolomide	24	9	6.6	11.4	36.0 ± 9.6	<0.001
Bevacizumab	13	9	6.9	11.1	30.8 ± 12.8	
Surgery ± adjuvant	15	13	1.6	24.4	53.3 ± 12.9	
Supportive care	20	2	0.7	3.3	5 ± 4.9	

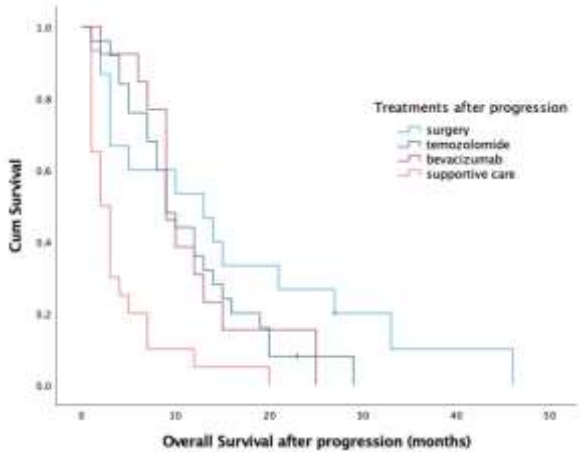


Figure 1. Survival curves of treatment groups after progression

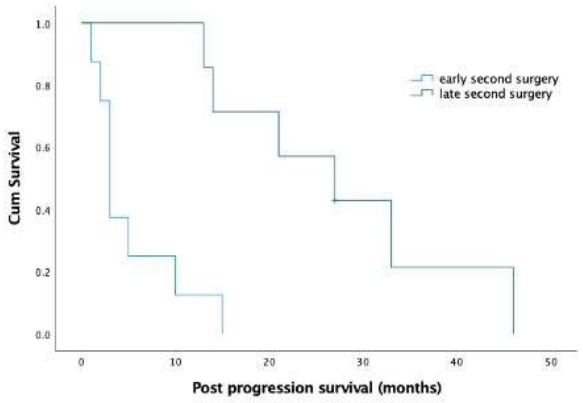
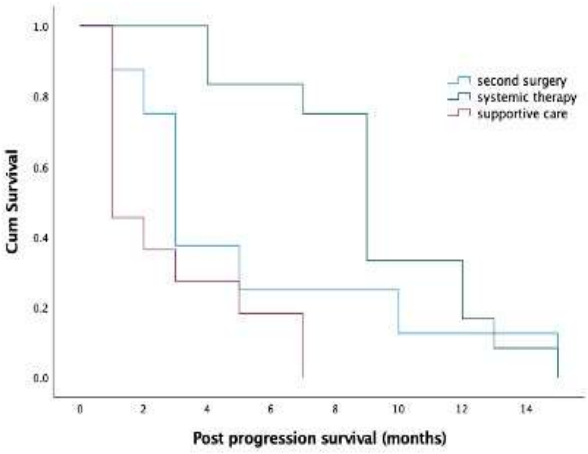


Figure 2: Kaplan Meier curves of surgery in early vs late progression

In multivariable analysis with factors with a p value of <0.05, we found progression time 9 months or less (p<0.001) and the use of supportive care after progression (p<0.001) as negative prognostic factors. Risk of death after progression was increased by a coefficient of 2.8 (95% CI: 1.6-5.0) in individuals who progressed ≤9 months in comparison to those who progressed later than 9 months. Re-operation (HR:0.20, 95% CI: 0.10-0.44), TMZ treatment (HR:0.37, 95% CI: 0.20-0.69) and BVC treatment arms (HR:0.26, 95% CI: 0.12-0.55) were associated with a lower risk of death after progression in comparison to supportive care arm (Figure 1).

Patients who underwent re-operation with or without adjuvant treatment had the longest



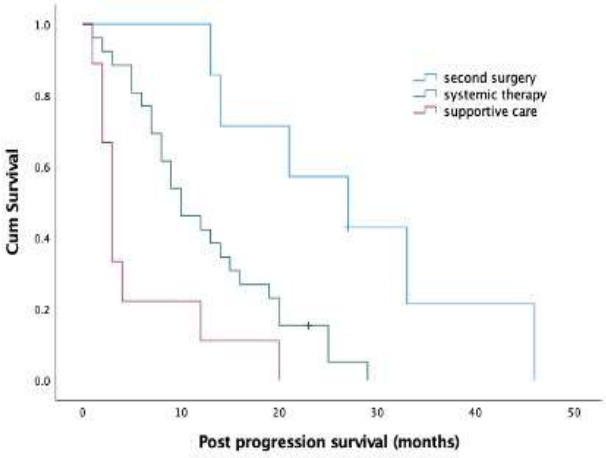


Figure 3: Kaplan Meier curves of treatments in a) early and b) late progression

PPS times (median 13 months, 95% CI: 1.6-24.4). However, when we consider the timing of re-operation; there was a significant PPS difference between early and late resections (median 3 months (95% CI:2.1-3.9) vs median 27 months (95% CI:11.6-42.4), p:0.001) (Figure 2).

In case of early progression; systemic therapy had a longer PPS than supportive care (median 9 vs 1 months, p<0.001). However there was no significant difference between re-operation and supportive care (median 3 vs 1 months, p=0.143) (Figure 3A).

In case of late progression; re-operation provided rates of PPS than systemic therapy (median 27 vs 10 months, p=0.005) and

supportive care (median 27 vs 3 months, p<0.001) (Figure 3B).

Discussion

Progression is inevitable after initial treatment of GBM. Today, however, there is no standard treatment for pGBM. In daily practice, if the clinical status of patient is suitable, the first option in mind is re-operation. However, re-operation is a highly controversial issue which has not been proven by randomized clinical trials. Analysis of 19 phase 2 studies by the North American Brain Tumor Consortium revealed no benefit of additional operations [9]. Nevertheless, the vast majority of literature supports the benefit of re-operation in pGBM [10-12]. A recent systemic review and a meta-analyse also advocates re-operation for progression [7,13]. Moreover, multiple resections have been found to increase survival. Chaichana et al. reported overall survival of 6.8, 15.5, 22.4 and 26.6 months after resections 1, 2, 3 and 4th, respectively [14]. In contrast, a study conducted in TMZ era revealed that patients with multiple resections were much younger and had higher perpormance status. And once adjusted for age, the benefit of multiple resections was no longer significant [15]. Recent data from the DIRECTOR cohort conclude that the benefit of re-operation is only with the removal of complete resection of the contrast-enhancing lesion [16]. Another study suggested that neutrophil/lymphocyte ratio (NLR) is a prognostic factor for PPS. The authors reported a median PPS of 9.7 months for NLR ≤ 4 and 5.9 months for NLR > 4 [17].

All these studies demonstrate the need for appropriate patient selection for re-treatments. Two studies analyzing data from phase 1 and 2 trials in North America and Europe reported that prognostic factors affected survival outcomes more than treatment modalities [18, 19]. Carson et al. identified age (≥50 vs. <50), 10 points increase in KPS and corticosteroid use in their recursive partitioning analysis for GBM. Gorlia et al. found World Health Organization (WHO) performance status, baseline steroids, tumor size (≤42mm vs. >42mm) and number of target lesions (1 vs. more than 1) as prognostic calculators. These prognostic factors are important to decide which treatment is avaliable for which patient. Several other studies also suggest that preoperative KPS scores are associated with higher OS time [20]. We have inadequate data of KPS at the time of first progression. However, an initial score higher than 70 to be correlated with better PPS trend after progression.

In this study, progression time greater than 9 months and re-treatment instead of supportive care were associated with longer survival after progression. Systemic therapies have similar PPS (9 and 10 months) when administered in case of early or late progression. However the benefit of re-operation reversed when it was incorporated into early progression. McGirt et al. emphasized the importance of gross total resection in both primary and secondary resections and found the benefit of a second surgery after 12 months of primary resection, but not earlier [21]. In a radiosurgery trial from Korea, radiosurgery and TMZ had a 15.5 month survival after progression. They also concluded that patients who progressed late, had better survival rates [22]. Conversely, Nava et al. and Ringel et al. found no prognostic effect of the progression time [23, 24]. The first found no benefit of reoperation in patient cohorts both before and after 2005. In addition, there was no 9-month threshold difference for resection results. However lower OS in the study (11.7 before TMZ and 12.9 after TMZ) may affect the outcomes. The latter found high survival rate (25 months), and good response to re-operation not dependent on the progression time. This may be due to the inclusion of well-selected patients with high KPS (median 90%) and low rapid progression rate (19% of patients had progression earlier than 5 months).

A recent study by Goldman et al pointed out the importance of time-dependent methodology for oncologic treatments [25]. They found that, re-operation was associated with a lower risk of death when timing was ignored (HR:0.62, 95% CI: 0.43-0.90, p=0.01). However, re-operation was associated with a higher risk of death after timing was taken into account (HR:2.19, 95% CI:1.47-3.28, p<0.001). This was also confirmed in a meta-analysis by Zhao et al. and a more recent single-instution study [7,26].

The evidence about the outcomes of surgical intervention are commonly from retrospective investigations. We need further researches to come to a conclusion and obtain higher-level evidence on the impact of surgery. The rate of re-operation in previous studies is 10-30%. More recent studies have reported higher rates of re-operation, possibly with improved surgical technics. However, care should be taken when making decisions in rapidly progressive cases. Second surgery can be considered in young patients with good performance status and progressive disease location that can be safely resectable in non-eloquent brain area. It helps relieving of symptoms quickly and may serve a better quality of life. In addition, information about the histopathological features of progressive disease may shed light on new therapeutic pathways.

Recently, improvements in radiotherapy setting allows a secondary radiation in the selected pGBM. A variety of re-irradiation studies have shown results comparable to other treatment modalities. Skeie et al. reported 12-month survival with radiosurgery [27]. Two studies from North America reported that 11-month survival with re-irradiation and no benefit of additional surgery before or after hypofractionated stereotactic radiation therapy [5, 28]. In our study population, only 2 patients were re-irradiated. This may be a result of primarily consideration of re-operation and systemic treatments and keeping radiation for residual tumors. However, the worsening of the patients' symptoms led to a decrease in the radiological detection rate of second relapses. Irradiation can be a good non-invasive option in both early and late progression.

Bevacizumab is a vascular endothelial growth factor inhibitor. We observed that BVC has similar efficiency with other treatment options. It may provide a better quality of life, particularly in early progression and when high doses of corticosteroid needed.

Our study has various limitations, one is that while chemotherapy efficacy has been shown to be dependent on the methylation of the promoter for MGMT, we did not have results for all of our patients and thus, we evaluated chemotherapy effectiveness without taking this factor into account. Similarly, IDH-1 status was unknown in half of the patients. Further studies will clarify the role of underlying molecular profiles in the pGBM treatment setting. Another limitation is the presence of combination therapies after progression, making it difficult to assess the efficacy of each. Finally, although some studies proposed 6-month progression free survival is a critical end point for evaluating the effectiveness of treatment [29], we were only able to prove a second relapse in 16 patients, and used PPS in this comparison.

Conclusion

In conclusion, Glioblastoma is a tumor with dismal survival even though efforts to increase treatment options and their effectiveness are being made. Standard treatments in progressive GBM are lacking. Survival after progression is considered to be shorter than a year and proper patient selection is crucial when deciding to proceed re-treatments. In particular, re-operation may not be a viable option for early progression, and should be discussed in multidisciplinary boards.

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Original Article

The Impact of the COVID-19 Pandemic on Head and Neck Cancer Practice –A Tertiary Health Care Center Experience

COVID-19 Pandemisi Sürecinin Baş Boyun Kanserleri Pratiğine Etkisi –Üçüncü Basamak Sağlık Merkezi Deneyimi

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) has changed practice patterns of head and neck oncology services, as well as other service areas of health care. This study aims to describe the impact of COVID-19 on the services of the division of otorhinolaryngology, head and neck surgery at an academic tertiary referral hospital specialized in oncology. This is a single-center descriptive study conducted within the otorhinolaryngology department of a tertiary health care institution, which mainly provided service to oncological cases during the pandemic.

Methods: Data of cases over 18 years old on the numbers of outpatient visits, hospitalizations, otorhinolaryngological surgeries, and indications were obtained from March 1 to May 15 for 2019 and 2020 from hospital information management system. Data on preoperative test results of asymptomatic patients for COVID-19, going through for head and neck oncological surgeries were obtained from the same system.

Results: There is a decrease in the total number of outpatient visits in 2020 compared to 2019. (16814 vs. 7108, 57.7%). The numbers of hospitalizations and surgeries related to head and neck malignancies were increased despite the decrease in the total number of hospitalizations (278 vs. 129, $p < 0.001$) and in the total number of surgeries (231 vs. 111, $p < 0.001$). One of the 88 preoperative COVID-19 tests of asymptomatic patients was positive. No member of the staff got infected.

Discussion and Conclusion: Although there is a decrease in the number of patients in the 2020 period, the increase in the qualitative characteristics of the head and neck oncological procedures performed causes an increase in the difficulties / risks that health professionals face even though they do not work for pandemic services.

Keywords: Pandemics, Otolaryngology, COVID-19, Surgical Oncology, Health Care

ÖZET

Giriş ve Amaç: Koronavirüs hastalığı-2019 (COVID-19), sağlık hizmetinin diğer alanlarında olduğu gibi, baş ve boyun onkolojisi hizmetlerinin uygulama düzenlerini değiştirmiştir. Bu çalışma, COVID-19'un, onkolojide özelleşmiş üçüncü basamak bir akademik sağlık kurumunun Kulak Burun Boğaz, Baş ve Boyun Cerrahisi Bölümü hizmetleri üzerindeki etkisini açıklamayı amaçlamaktadır. Çalışmamız, pandemi sürecinde onkolojik olgulara hizmet veren üçüncü basamak bir sağlık kurumunun Kulak Burun Boğaz bölümünde yürütülen tek merkezli tanımlayıcı bir çalışmadır.

Yöntem ve Gereçler: Hastane bilgi yönetim sisteminden 2019 ve 2020 yıllarının 1 Mart-15 Mayıs tarihleri arasında 18 yaşından büyük olguların poliklinik başvurusu, hastaneye yatış ve kulak burun boğaz ameliyatları sayıları ile yatış ve ameliyat endikasyonlarına ilişkin veriler elde edildi. Baş ve boyun bölgesi onkolojik cerrahisi planlanan asemptomatik preoperatif hastaların COVID-19 test sonuçlarına ilişkin veriler aynı sistemden elde edildi.

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Bulgular: 2020'de poliklinik başvurularının toplam sayısında 2019'a göre azalma saptandı. (16814'e 7108, %57.7). Toplam hastaneye yatış sayısı (278'e 129, p <0.001) ve toplam ameliyat sayısındaki (231'e 111, p <0.001) azalmaya rağmen, baş ve boyun kanserleri nedeniyle olan ameliyat ve hastaneye yatış sayıları arttı. Asemptomatik hastaların ameliyat öncesi 88 COVID-19 testinden biri pozitifti. Bölüm personelinden enfekte olan kişi olmadı.
Tartışma ve Sonuç: 2020 döneminde hasta sayısında azalma olmakla birlikte baş ve boyun onkolojisine yönelik olarak uygulanan işlemlerin niteliksel özelliklerinde artış olması, sağlık profesyonellerinin pandemi hizmetleri için çalışmadığı durumlarda dahi karşı karşıya olduğu zorluk / risklerde artışa neden olmaktadır.

Anahtar kelimeler: Pandemi, otolaringoloji, COVID-19, Cerrahi Onkoloji, Sağlık hizmeti

Introduction

Coronavirus Disease – 2019 (COVID – 19) is a global problem and named as a pandemic by the World Health Organization on March 11, 2020. Since the very first cases of the disease in China, it is compared to the SARS (Severe Acute Respiratory Syndrome) and the MERS (Middle East Respiratory Syndrome) pandemics but it was clearly seen that the world is facing to a much bigger problem affecting all medical, social and economic issues.

Pandemics are periods in which the emergency and compulsory services that constitute the basics of health services should be prioritized. After the first COVID – 19 cases were reported, it was recommended that all hospitals review their pandemics plans, and to stratify urgent, non-delayable, and elective cases for each branch [1]. Head and neck cancer related procedures are prioritized by otolaryngology departments across the world. Clinical experiences provided in pandemics periods are also very valuable in terms of regulation of health services. Otorhinolaryngology units, providing either outpatient or inpatient services, were the first line to be affected by this pandemic because of the transmission way of the disease[2]. In the multimedia era, which is a main component of globalization, both patients and healthcare workers were getting information more quickly than the spread of the disease and this situation changed some practice patterns [3].

Herein, we discuss the early effects of the COVID-19 pandemic on our otorhino-laryngology service practice. Our institution is specialized in oncology long before the pandemic. The primary objectives were to examine the quantitative changes in outpatient visits, hospitalizations, and performed surgeries over the first months of the virus's impact within Turkey, in comparison with the prior year, paying extra attention to head and neck cancers. Secondarily, we analyzed trends of mentioned parameters in weekly changes.

Materials and methods

Setting

This was a retrospective, single-institution study conducted within the entirety of the department of otorhinolaryngology at a tertiary health care centerspecialized in oncology. The institution is an academic center that also includes community clinics. Cases requiring major surgeries are widely referred from all over the country, but also general otorhinolaryngological services provided to a city which has a population of near 6 million people. This study was approved by the Local Committee on Ethics (No. of meeting: 2021-01/937, January 13, 2021).

Period and Data

The first COVID-19 case was reported on March 11, 2020, in the country but a change of practice patterns, and postpone of elective surgeries on patient demand was seen before

that. Elective surgeries were temporarily halted by March 17, 2020, after the formal letter from the Ministry of Health.

As the focus of the study was on COVID-19's impact on otorhinolaryngology services and especially head and neck cancers, we queried data of from March 1 to May 15, 2020 (the 2020 period). For a comparison group, we queried the corresponding period from the previous year—March 1 to May 15, 2019 (the 2019 period). Dates were grouped into weeks 1–11 for each period from March 1, every seven days consisting of a week.

All patients attending the department aged over 18 years old (all database population) was included to minimize potential sources of bias. With the approval of the Local Committee on Ethics, writers had access to the database of the hospital information management system (HIMS) for data of all patients consisting date, diagnosis, performed procedures, results of preoperative tests.

Number of all outpatient visits within the otorhinolaryngology division were obtained from the HIMS. Detail of indications (hospitalizations and surgeries) were obtained fromHIMS database search with ICD-10 (International Classification of Disease) codes. Detailed data of performed surgeries were obtained by hospital information management system database search with Health Application Notification (HAN) codes. Data of surgeries were cross checked by departments operation registry book.

Hospitalizations were distributed to five major indication groups. The first group consists of patients with planned elective surgeries. The second group was hospitalizations before planned surgeries for head and neck cancers. The third group consists of patients who need hospitalization for advanced diagnostic procedures or biopsy (direct laryngoscopic examination, lymph node excision, imaging techniques requiring inpatient evaluation before a procedure, etc.). The fourth group of patients was hospitalized for debridement or drainage of infections like a peritonsillar abscess, chronic osteomyelitis of the jaw after radiotheraphy, etc. The fifth group of patients had otorhinolaryngological emergency conditions requiring medical or surgical treatment, like desaturation because of upper respiratory tract obstruction (common presenting symptom of head and neck cancers), post-tonsillectomy bleeding, or sudden sensorineural hearing loss.

Surgical procedures were distributed to four groups. The first three groups are the same as hospitalization groups. The fourth and fifth groups were combined for statistical purposes: surgeries for debridement and emergencies consist the fourth group.

Service providing specialists were general otorhinolaryngologists as subspecialties are not yet established in the country. All have at least 5 years of experience in head and neck surgery.

Statistical Analysis

Chi-square ($\chi 2$) test was used to compare categorical data. Statistical analysis was performed via IBM SPSS for Windows version 26.0 and significance was set at $p<0.05$.

Results

Outpatient services

Between March 1 and May 15, 2019; 16814 visits are completed within the otorhinolaryngology division. This was distributed to 14 providers: seven otolaryngologists (one professor, two associate professors, four specialists), four residents (all general practitioner, medical doctors), one speech and language pathologist, and two audiologists. In the COVID – 19 period (March 1 – May 15, 2020) 7108 outpatient visits were completed by the same providers. There is more than half

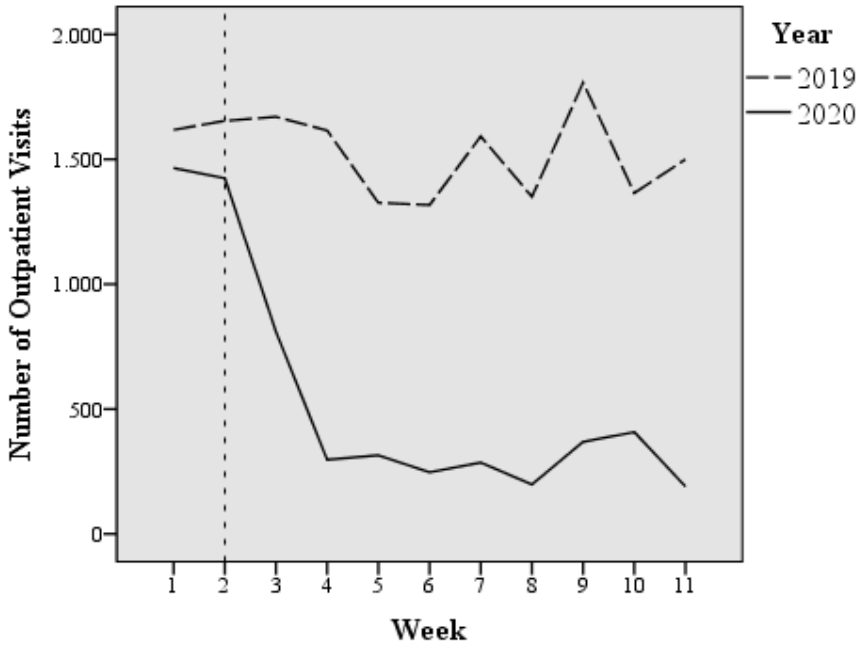

Figure 1. The weekly breakdown of completed outpatient visits in the 2019 and 2020 periods. Note that the first case was seen on March 11, 2020 (week 2, vertical dotted line).

decrease (57.7%) of closed outpatient visits (Figure 1).

Hospitalizations

Between March 1 and May 15, 278 patients were hospitalized in 2019. Between March 1 and May 15, 129 patients were hospitalized in 2020. On chi-square analysis, a smaller number of patients were hospitalized in 2020 period as compared with 2019 period. (129 vs 278, $p < 0.001$). The decrease in the number of hospitalized patients is obviously seen on weekly basis (Figure 2). Distribution of hospitalization indications has also changed by the pandemic, mainly because of elective cases (Figure 3).

Surgical procedures and preoperative tests

Between March 1 and May 15, 231 otorhino-laryngological surgeries were performed in the operating room in 2019. Between March 1 and May 15, 111 otorhinolaryngological surgeries were performed in the operating room 2020. On chi-square analysis, there is a significant decrease in the number of operations in 2020 as compared with the number of operations is in 2019 (111 vs 231, $p<0.001$). This decrease can be seen in the weekly breakdown of surgeries (Figure 4).

Surgical indications were also changed. In 2019 most of the surgeries were elective (71.9%), whereas surgeries for malignancies take the first place (40.5%) in 2020 (Figure 5).

The most common surgical procedure in the 2020 period was a major head and neck cancer surgery:14 neck dissections of eight patients (five total laryngectomy + bilateral neck dissection, two hemiglossectomy + unilateral neck dissection, one lower lip midline carcinoma excision + bilateral anterolateral neck dissection) were performed. Also, one patient had neck dissection within the COMMANDO Procedure (COMbined MAndibulectomy and Neck Dissection Operation) and pectoralis major muscle skin flap repair. Another patient with oral cavity (gingiva-mandible) carcinoma diagnosed just

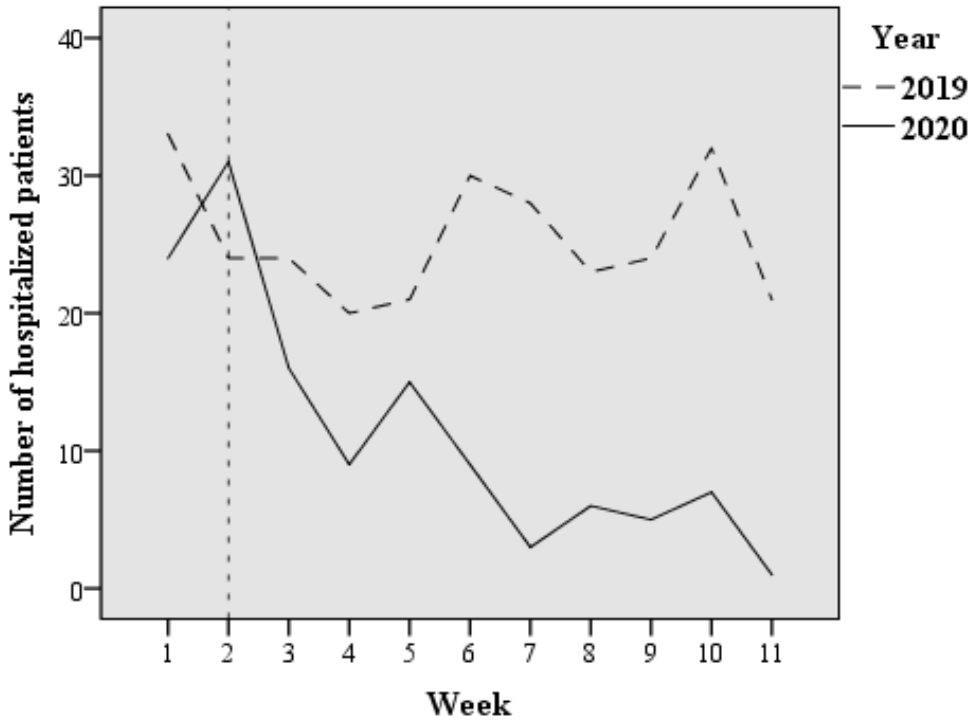

Figure 2. The weekly breakdown of hospitalizations in the 2019 and 2020 periods. Note that the first case was seen on March 11, 2020 (week 2, vertical dotted line).

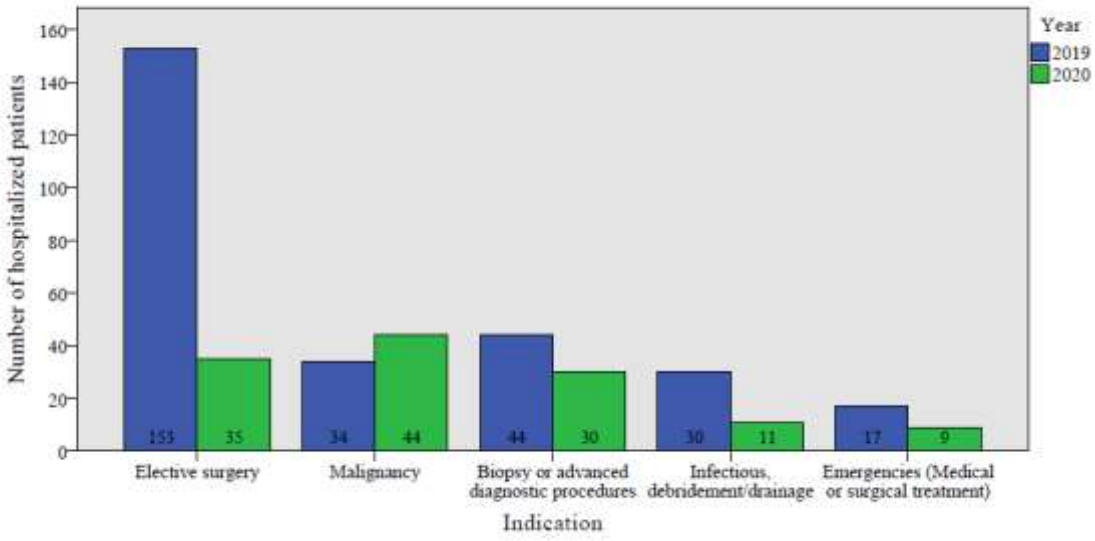

Figure 3. Distribution of hospitalization indications in the 2019 and 2020 periods.

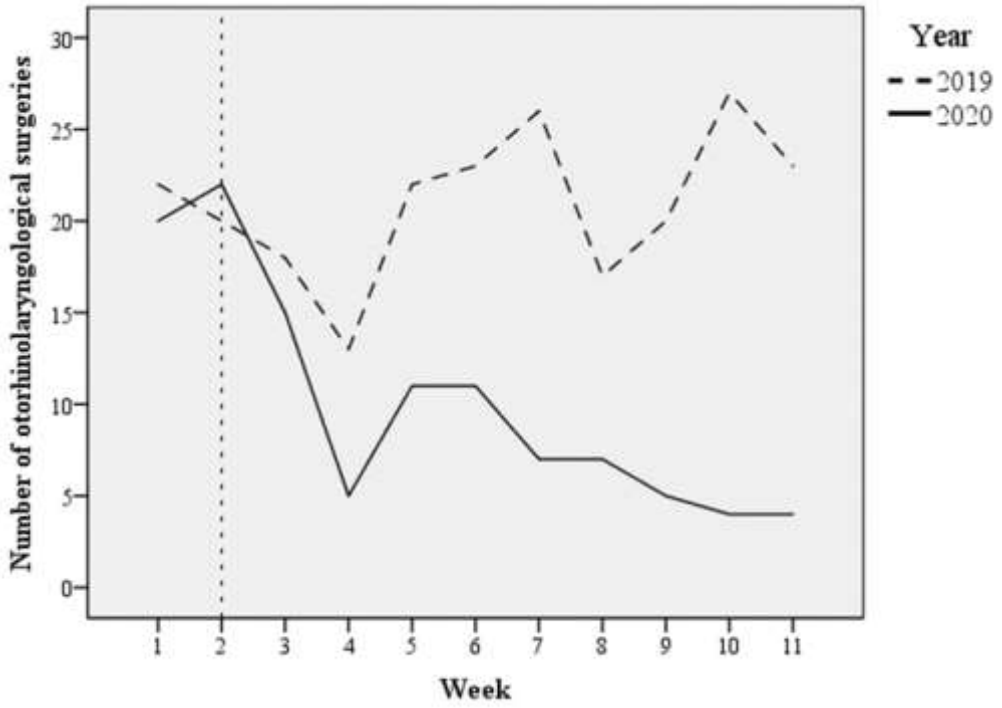

Figure 4. The weekly breakdown of otorhinolaryngological surgeries performed in the operating room in 2019 and 2020. Note that the first case was seen on March 11, 2020 (week 2, vertical dotted line).

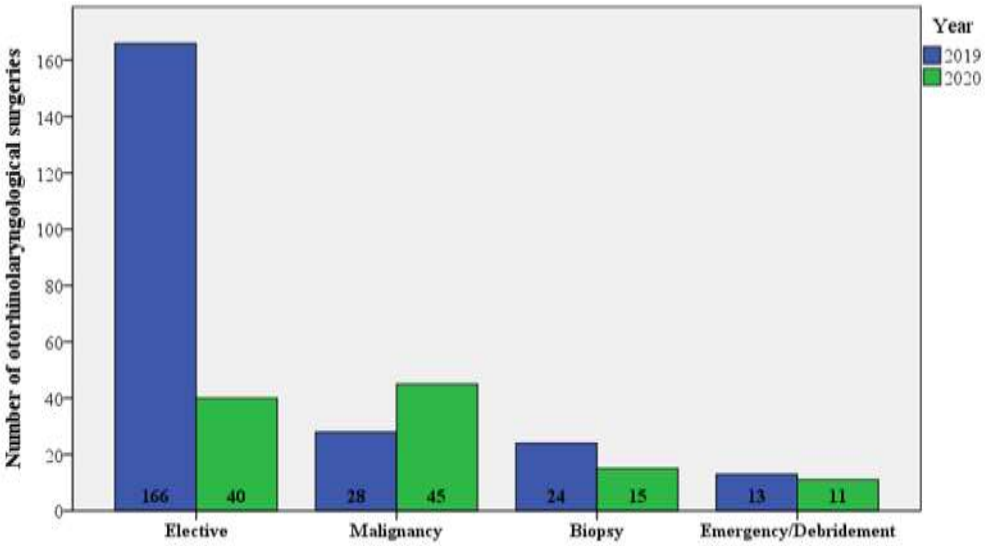

Figure 5. Distribution of indications for surgery in the 2019 and 2020 periods. 40 elective surgeries were performed between March 1 and March 17, 2020

before the outbreak, 10 years after tongue carcinoma treatment on the same side, had lateral segmental mandibulectomy for tumor resection and pectoralis major muscle skin flap repair.

The second most common surgical procedure in the 2020 period was direct laryngoscopy and laryngeal biopsy of 11 patients with suspected malignancy. Two of them had a tracheotomy in the same session. Besides, one

patient with respiratory distress and one patient with prolonged intubation received a tracheotomy.

Total laryngectomy for five patients, partial / hemiglossectomy due to tongue carcinoma (+/-neck dissection) for four patients was performed in the 2020 period. Other surgeries include resection and repair of facial skin cancers, debridement of invasive fungal rhinosinusitis in the same period.

To protect staff and to ensure continuity of healthcare, preoperative polymerase chain reaction (PCR) testing for COVID-19 was provided for asymptomatic head and neck cancer patients by the ninth week of the 2020 period, after discussion with the institutional COVID-19 committee. Samples for PCR tests were collected by nasopharyngeal/ oropharyngeal combined swabs. A total number of 12 PCR tests were performed, 1 resulted positive. The staff members involved with the patient were isolated at home with symptom monitoring, all had PCR tests done on the fifth day, all results were negative. After the start of the "normalization period" (June 1, 2020), to July 29, 2020, an additional number of ,76 PCR tests were performed and all resulted negative. The latter 76 test were performed in consistency with the recommendation of the Ministry of Health, for patients with planned undelayable major surgeries.

Discussion

The COVID – 19 pandemics has changed the structure of health care globally and continues to do so with every piece of knowledge obtained about it [4]. Using different platforms, physicians are trying to get information about each other's practices to give the best decisions about the model of service, sometimes requiring new methods for sharing updated knowledge [5]. The knowledge about the virus, ways of treatment, and protection is updating and characteristics involving otorhinolaryngology are widely discussed[6] but we have to put this information into practice.

An outbreak period caused by an aerosol-borne disease, people with flu-like symptoms are stratified as "high-risk patients"[7]. This group of patients constitutes an important part of otorhinolaryngology outpatient visits. Another important problem is that COVID-19 cases can be asymptomatic at a rate of 5-80%[8]. Therefore, high risk is not only limited to pandemic outpatient services but also directly related to the nature of the examinations or procedures performed.In all units of the department performing outpatient otolaryngological services, personal protective equipment (PPE) consist of N95/FFP2 mask plus surgical mask, goggles/ visor, bonnet, double gloves and disposable gowns recommended by local association of otolaryngology head and neck surgery [9] are routinely used for procedures described as "close contact" by the "COVID-19 Advisory Committee of Ministry of Health of Turkey".

During the 2020 period, the number of outpatient visits is decreased as expected and observed in pandemics [4,10,11]. This may be both from the reduction of appointments and hesitation of patients to go to a hospital building during the outbreak. Despite the dramatic decrease compared to the year before, an average of 120 outpatient visits per day are completed, mainly for head and neck cancers. Although for the first days we had some organizational issues, we did not face any shortage of appropriate PPE recommended by national or international guidelines [9,12]. If the PPE mentioned above fits the healthcare worker properly, hypercapnia and associated symptoms develop after a while. This situation during the outpatient service causes additional strain, breaks between visits should be maintained. Transparent booths for patient examination were used when appropriate. Other valuable precautions to ensure the protection of staff is

flexible working order which also prevented cross-contamination and screening of all patients who were asked to wear surgical masks before the visit. All patients were screened for COVID-19 by measuring body temperatures without contact and filling the inquiry form prepared by the Ministry of Health, even if they are asymptomatic. If any patient has susceptible signs or symptoms, referral to the pandemic outpatient clinic is provided. As no member of the staff got infected during the period, these precautions seem to be protective for the outpatient clinic staff.

Most hospitalizations were for surgical purposes both in the 2019 and 2020 periods. The most prominent cause in the dramatic decreases in the number of hospitalized patients and in the number of otorhino-laryngological surgeries was the reduction of elective indications. These are common findings for pandemics [10,13]. By the fourth week of the 2020 period, there is a slight increase in the number of hospitalizations and surgeries. With a decision of the provincial health directorate, COVID-19 positive patients were cohorted to other specified hospitals in the city, and cases from those hospitals referred to our institution for major otorhinolaryngological surgeries, most of which are head and neck cancer. So, as a difference from other institutions' experiences [14], there was an increase in the number of surgeries for malignancies in the 2020 period. This caused extra effort of the staff with mentioned PPE but in the end, patients received appropriate treatment on time with no extra complications.

Our oncological practice also changed by the pandemic. As COVID -19 causes increased mortality in post-operative period[15], some head and neck carcinoma cases were referred to Radiotherapy Department directly instead of performing surgery, if the expected treatment success rate is close for each option. Surgical treatment of squamous cell cancers of head and neck is recommended to continue in the pandemic period if postponing causes progression in the stage of the disease or results more aggressive approach [16]. Head and neck cancer operations involve upper aerodigestive tract surgery, causing extra aerosol production which increases the risk of viral exposure. A significant proportion of patients may be asymptomatic so temperature measurements and symptom inquiries may not work for these cases. After transnasal surgery of an asymptomatic case from China, over 14 health care workers have reported being infected [17]. Since the normalization period has started on June 1, 2020, even asymptomatic cases are having PCR tests before major surgeries in our institution, after the recommendations of the national advisory board. Appropriate filiation of the case with the isolation of the health care workers involved to the case may save money and health by performing preoperative PCR testing of asymptomatic patients. But in our experience (from March 1, 2020, to July 29, 2020), a total number of 88 preoperative PCR tests for asymptomatic patients were performed, one resulted positive. No member of the otolaryngology operating room staff developed symptoms or had positive PCR test results up to July 29, 2020. More researches should be done on the concept of preoperative PCR testing, as we are performing surgeries with the mentioned PPE even the PCR test is negative.

As mentioned before appropriate use of PPE by the entire otorhinolaryngological service providing team is very important, even with negative PCR results. We did not use powered air-purifying respirator (PAPR) or disposable overalls for patients with undetermined COVID-19 status. Performing major oto-rhinolaryngological surgeries with overalls + PAPR for hours may be an extra physical burden to the surgeon besides the costs.

Research should be conducted on this issue.

Because of the feature of the hospital mentioned above, we have no findings to present on proven COVID-19 positive cases. Another limitation of the study is the lack of telemedicine data which is still a matter of debate for some otorhinolaryngological services.

Conclusion

In the current study, the COVID-19 outbreak experience of a tertiary otorhinolaryngology head and neck surgery department is explained. In general, the number of patients by terms of outpatient visits, hospitalizations, and performed surgeries are reduced but the qualitative features of the procedures performed are increased, mainly as a result of the increase in head and neck malignancy related procedures. Because of these changes in practice patterns, the difficulties/risks that otorhinolaryngology healthcare professionals are facing are increased in the 2020 period compared to the 2019 period.

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Original Article

Accuracy of Preoperative Computed Tomography for Lymph Node Status Screening in Colon Cancer

Kolon Kanseri Hastalarında Lenf Nodu Durumunu Görüntülemede Preoperatif Bilgisayarlı Tomografinin Doğruluğu

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ABSTRACT

Introduction: Our aim is to determine the value of a pre-operative Computed Tomography (CT) scan for the assessment of lymph node status in patients diagnosed with colon cancer by comparing between radiological N-stage and histopathological N-stage.

Methods: After approving by local ethics committee, an experinced radiologist reviewed all pre-operative CT scans of patients diagnosed with colon cancer retrospectively, between January 2014 and December 2018. The CT scans were examined for any signs of regional lymphatic spread which was defined as lymph nodes exceeding 1 cm, clusters of ≥3 lymph nodes or a combination of the two. The results were compared with the histopathological N-stage. The diffrences in comparison were eveluated statistically and positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity and accuracy were calculated.

Results: We included 184 patients in our study. The statistical values of PPV, NPV, sensitivity, specificity, and accuracy of detecting regional lymph nodes metastases were 65.6%, 75%, 58.3%, 80.3% and 71.7%, respectively. The assessment of lymph node status with CT scans resulted in a moderate sensitivity, specificity and accuracy for both subgroups, defined as emergency and tumor localization subgroups.

Discussion and Conclusion: Although our study group is relatively large and homogeneous compared to previous studies, the obtained results in the evaluation of patients with colon cancer with preoperative CT does not seem to be satisfactory. Before making the treatment decisions according to the appearance of lymph nodes in colon cancer patients on CT images, the diagnostic accuracy needs strong improvement, such as thinner axial slices and three-dimensional reconstruction methods.

Keywords: Colon cancer, Lymph nodes status, Computed tomography, Preoperative staging

ÖZET

Giriş ve Amaç: Çalışmamızda kolon kanseri tanılı hastalarda radyolojik ve histopatolojik lenf nodu evresini kıyaslayarak preoperatif bilgisayarlı tomografi (BT) ile taramanın lenf nodu durumunu belirlemedeki değerini belirlemeyi amaçladık.

Yöntem ve Gereçler: Ocak 2014 ve Aralık 2018 tarihleri arasında kolon kanseri tanısı almış olan hastaların preoperatif BT taramaları alanında deneyimli bir radyolog tarafından geriye dönük olarak

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incelendi. Görüntülerde bölgesel lenf nodu yayılımının göstergesi olarak 1cm'den büyük lenf nodu, ≥3 kümelenmiş lenf nodları veya her ikisinin de mevcut olması kabul edilmiştir. Sonuçlar histopatolojik lenf nodu evresi ile kıyaslandı. Ardından, pozitif prediktif değer (PPD), negatif prediktif değer (NPD), sensitivite, spesifisite ve doğruluk değerleri hesaplandı.

Bulgular: Çalışmamıza 184 hasta dahil olmuştur. Bölgesel lenf nodu metastazının tespitinde tespit edilen PPD, NPD, sensitivite, spesifisite ve doğruluk değerleri sırasıyla %65.6, %75, %58.3, %80.3 ve %71.7 idi. Acil ve tümör yerleşimi gibi alt gruplarda BT ile lenf nodunun değerlendirilmesinde ise BT'nin orta düzey sensitivite, spesifisite ve doğruluğa sahip olduğu görüldü.

Tartışma ve Sonuç: Çalışmamız daha önceden yapılmış olan çalışmalara göre nispeten daha çok hasta içermesi ve daha homojen yapıda olmasına rağmen, kolon kanseri hastalarının preoperatif BT ile lenf nodu durumunu değerlendirilmesinde tatmin edici sonuçlara ulaşılamamıştır. BT görüntülemedeki lenf nodunu görünümüne göre tedaviye yön vermede tanısal doğruluğu arttıracak ince aksiyal kesitler ve üç-boyutlu rekonstruksiyon yöntemleri gibi güçlü gelişmelere gereksinim vardır.

Anahtar Kelimeler: Kolon kanseri, Lenf nodu durumu, Bilgisayarlı tomografi, Preoperatif evreleme

Introduction

Colorectal Cancer (CRC) is the most common diagnosed gastrointestinal neoplasm in western world. Approximately 70% of cases are located to colon and the treatment for resectable CRC is curative surgery with adequate lymph node (LN) dissection.

Preoperative chemoradiotherapy is the approved treatment to make smaller the tumor size and prevent local recurrences for high-risk rectum cancer [1]. American Joint Committee on Cancer (AJCC) recommends adjuvant chemotherapy for stage 3 colon cancer [2]. Colorectal surgeons have began to prefer neoadjuvant chemotherapy (NAC) for high-risk colon cancer preoperatively to prevent local recurrences after surgery; however, determining the appropriate patient for neoadjuvant therapy became more important [3]. Endorectal ultrasonography (EUS) and magnetic resonance imaging (MRI) are used to stage rectum cancer patients for NAC, but EUS is not appropriate for colon neoplasms, and also positron emission tomography (PET) and MRI have a low sensitivity [4,5]. Therefore, CT seems to be the only imaging modality to determine the distant metastasis and LN status and to select appropriately colon cancer patients for NAC. We aimed to reveal the effect of preoperative CT in diagnosing the LN status in colon cancer.

Material and Methods

After the local ethics committee approval (25.12.2017-44/15), the hospital records of elective and emergency curative surgery performed patients between January 2014 and December 2018 were analyzed retropectively. Rectum neoplasms, NAC performed patients, synchronous neoplasms, stage 4 neoplasms, palliative surgery (bypass or enterostomy) performed patients, recurrent colon neoplasms, less than 12 LN dissected patients and CT images missing patients were excluded from the study. CT images were examined by a single radiologist (YA) with more than 10 years of abdominal CT experience by knowing only the primary tumor localization of the patient. Radiological examination defined positive LN as; diameter >1 cm regional LN and\or count of ≥3 clustered regional LN. Intravenous (IV) and oral contrast agents were performed for all elective cases, and only IV contrast agent was performed for emergency cases.

The abdomen of the patients was scanned from diaphragm to pelvis with Toshiba Alexion 16 slice CT Scanner. Iohexol 300 mg I/mL was administered intravenously according to the weight of the patient with an

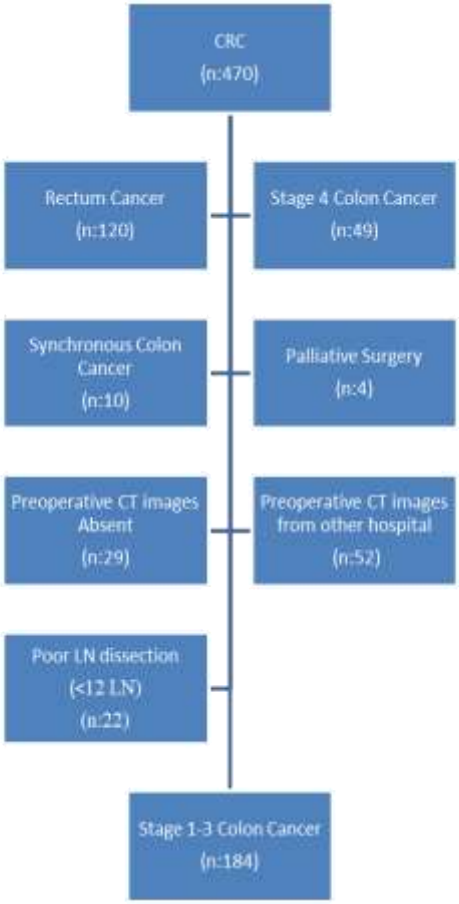

Figure 1. Inclusion and exclusion criteria

Imaxeon syringe. All CT scans were viewed on 3 mm axial sliced images. The maximum short axis in the axial plane was measured. All captured images were recorded in the hospital's PACS system.

Histopathological examinations of resected colon specimens were performed compatible with standard references, and LN were isolated by the only dissection, without oil cleaning techniques. The differentiation of pN1 and pN2 was made compatible with TNM classification, 8th edition, in subgroup analysis [6].

The data analyses were done with IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY, USA) package software. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of CT were analyzed.

Table 1. The patients and neoplasm demographics

		N (%)
Stage 1-3 colon cancer patients		184
Gender		
	Male	110(59.7)
	Female	74(40.3)
Age		
	Median	66.0
	Range	38-91
Surgery		
	Emergency	53(28.8)
	Elective	131(71.2)
Localization		
	Right colon	56(30.3)
	Transvers colon	9(4.9)
	Left colon	31(16.8)
	Sigmoid colon	44(23.8)
	Rectosigmoid	44(23.8)
Tumor size		
	Median	4.7
	Range	2-13
T		
	T1	10 (5.4)
	T2	19 (10.3)
	T3	111 (60.3)
	T4	44 (23.9)
N		
	N0	112 (60.8)
	N1	55(29.8)
	N2	17 (9.2)
TNM		
	Stage 1	24 (13.0)
	Stage 2	87 (47.3)
	Stage 3	73 (39.7)

Results

A total of 470 diagnosed CRC patients were operated between January 2014 and December 2018. After exclusions, 184 TNM stage 1-3 colon cancer patients who had curative surgery, were included in the study (Figure 1).

The patients and neoplasm demographics were listed in Table 1. T3 + T4 tumours (155\184) were 84.2 % of the population and 39.1 % (N1+N2; 72/184) of cases had LN involvement (Table 1).

Table 2. Distribution of patients according to histopathological and radiological findings

		histopathological lymph node involvement		
		pLN+	pLN-	
preoperative CT images	rLN+	True positive(n): 42	False positive(n):22	PPV*: 65.6%
	rLN-	False negative(n):30	True negative(n):90	NPV**:75.0%
		Sensitivity: 58.3%	Spesificity: 80.3%	

pLN+: pathological lymph node+; pLN-: pathological lymph node-; rLN+: radiological lymph node+; rLN-: radiological lymph node-*positive predictive value,** negative predictive value

Table 3. PPV, NPV, Sensitivity, Specificity, and Accuracy

	PPV*	NPV**	Sensitivity	Specificity	Accuracy
Total (n:184)	65.6%	75.0%	58.3%	80.3%	71.7%
T1+T2 (n:29)	25.0%	84.0%	20.0%	87.5%	75.8%
T3+T4 (n: 155)	68.3%	72.6%	61.1%	78.4%	70.9%
Elective Surgery (n:131)	67.3%	70.7%	57.8%	78.3%	69.4%
Emergency Surgery (n:53)	60.0%	84.2%	60.0%	84.2%	77.3%
Right+Transvers Colon (n:65)	63.3%	80.0%	73.0%	71.7%	72.3%
Left+Sigmoid+Rectosigmoid Colon (n:119)	67.6%	72.9%	50.0%	84.9%	71.4%

*positive predictive value,** negative predictive value

A comparison of preoperative CT images with histopathological findings revealed true positive and negative, false positive and negative cases (Table 2). We found 42 true positives, 90 true negatives, 22 false positives, and 30 false negatives.

Diagnosis of malign LN's PPV, NPV, sensitivity, specificity and accuracy rates were; 65.6% (95% CI 55.5%- 74.4%), 75% (95% CI 69.22%-80%), 58.3% (95% CI 46.11%-69.8%), 80.3% (95% CI 71.7%-87.2%) and 71.7% (95% CI 64.6%-78.1%), respectively (Table 3).

Discussion

To date, there has been a tendency on multimodal therapies, including chemotherapy, radiotherapy, and surgical procedures to surgery alone therapies for different colon tumor stages [7]. One of the conspicous alteration of these treatment modalities is the NAC for local advanced and LN involved colon neoplasms, which gains significantly increased survival rates [8]. NAC has become the gold standard treatment modality for the esophagus, stomach, rectum, and breast cancer; however, it has been arguable validity for colon cancer.

CT is widely used to evaluate the primary tumoral lesions and distant metastasis for preoperative staging, in addition LN metastasis could be diagnosed accurately by CT preoperatively. The previous studies stated that; the sensitivity of CT for T3 and T4 tumors was above 90% in the evaluation of pathological T [9], while the sensitivity for

detection of malignant LN ranged between 13% to 92% [10-12].

CT axial sliced images vary from 5mm-8mm to 10mm in different studies; thus, limitations and discussions in evaluating LN with CT are associated with these variable and wide-ranged results. Dighe et al. stated that results were better in determining the metastatic LN when the axial sliced was ≤5 mm with their metanalysis study [13].

The definition of metastatic LN's image on CT is variable in different studies. LN's diameter size >5 mm [14], size >8mm [15], size >1cm [16], ≥3 number of LN [16], and large LN with irregular contours [14] were defined as metastatic LN in some studies. However, inflammatory large-sized LN, metastatic small-sized, and non-clustering LN have revealed that it is misleading to use dimensional or morphological findings alone in the evaluation of metastatic LN [17].

Therefore, researchers have began to conduct studies using more than one findings in the evaluation of metastatic LN. Rolven et al. reported that the CT sensitivity was 85%, and specificity was 75% in detecting stage 3 colon cancer, using together with the internal heterogeneity and irregular LN border [18]. In another study where LN size >5 mm and\or irregular contours defined as metastatic LN, CT sensitivity and specificity were 64% and 53%, respectively [19].

Measuring the axial length of LN is another commonly used method for LN evaluation; however, an LN shorter than 1 cm in axial sequence may be longer than 1 cm in sagittal or coronal sequences. Kanamoto et al. suggested two or three-dimensional reconstruction methods to increase sensitivity, specificity, and accuracy rates in their studies [20], but these methods seems to be time-consuming. They also stated that in axial sequence, CT images using short\long axis ratio ≥0.8 to define metastatic LN raised sensitivity to 87% and specificity to 80% [20].

The low rate of intraabdominal adipose tissue complicates to distinguish the infiltrated tumor into the pericolonic adipose tissue from local LN metastasis, and it is accepted that evaluation of LN status with CT will be difficult for these patients. Acute obstructive colon neoplasms with over dilated colon segments will distort the CT images, leading LN evaluation. On the other hand, Sjovall et al. reported that there was no difference between CT evaluation of primary colon tumor and histopathological T and N depending on criterias such as age, gender, BMI, emergency surgery, and localization of the tumor [21]. We used only intravenous contrast for emergency surgery and both intravenous and oral contrast for elective surgery patients during CT. We also observed that there was no significant proportional difference in terms of lymph node evaluation between patients who underwent emergency and elective surgery. Likewise, there was no abnormal change according to the tumor location according to current study. According to our radiologic criteria, the assessment of LN status with CT resulted in a moderate sensitivity and specificity for both elective and emergent patients.

Preoperative CT has not been able to get desired results in the evaluation of LN. Once we defined the metastatic LN as diameter >1 cm and\or ≥3 clustered LN, 11.9% (22/184) patients would get unnecessary NAC and 16.3% (30/184) patients who were a candidate for NAC would not get NAC in our study. The T1+T2 subgroup analysis revealed that; PPV and sensitivity were significantly lower than the other groups. These results indicated that; it is necessary to determine the structural findings as well as the dimensional findings, even T status, and all findings together in determining the metastatic LN. Besides,

radiologic criteria for lymph node metastases on CT in colon cancer resulted in moderate specificity and sensitivity both in left sided and right sided disease.

The retrospective nature of the study, single radiologist examination, and the limited number of patients are the limitation of our study.

In conclusion, although CT has been performed via thinner axial sliced images and relatively more homogeneous groups compared to literature, the results we obtained in the evaluation of patients with colon cancer with preoperative CT are not satisfactory. In order to reach an improvement in universal definition and accuracy for the different treatment options, there is a need for further studies with a prospective, more significant number of patient groups, using thin axial sliced and three-dimensional reconstruction methods and examining more than one findings for colon cancer patients' pre-operative LN examination with CT.

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Original Article

Predictive Role of Blood Flow Characteristics in the Detection of Malignant Breast Lesions: A Prospective Study

Malign Meme Lezyonlarının Saptanmasında Kan Akışı Özelliklerinin Prediktif Rolü: Prospektif Bir Çalışma

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ABSTRACT

Introduction: The aim of this study is to investigate the predictive feature of lateral thoracic artery (LTA) and internal thoracic artery (ITA) power doppler ultrasound examination results of patients with malignant breast lesions.

Material and Methods: This is a prospective case-control study in which 47 patients with suspicious lesions detected by ultrasonography and mammography and diagnosed with pathologically invasive breast carcinoma between 2018-2020 were included in a tertiary hospital. The breast with invasive carcinoma and the intact breast of the same patient were evaluated with LTA and ITA by power doppler ultrasonography. Healthy breast was the control group.

Results: In the diagnostic statistical tests; the optimum cut-off value for LTA peak systolic flow (PSF) value is 19.6 cm/s, sensitivity of this value is 81.3% and specificity is 95.8%. The optimal cut-off value for LTA resistive index (RI) value was 0.805, sensitivity of this value was 91.7% and specificity was 95.8%. For the detection of invasive breast carcinoma in the contralateral breast, the sensitivity of ITA peak diastolic flow (PDF) below 8.35 is 77.1% and the specificity is 79.2%.

Conclusion: To the best of our knowledge, this study is the first publication to examine the flow patterns of internal ITA and LTA together. This study presents new quantitative diagnostic tests that can be used to detect breast cancer, are easily accessible, applicable, and have high sensitivity and specificity.

Keywords: Breast Tumors; Blood Flow Velocity, Doppler Ultrasound Imaging, Thoracic Arteries

ÖZET

Giriş: Bu çalışmanın amacı malign meme lezyonu olan hastaların lateral torasik arter (LTA) ve internal torasik arter (İTA) power doppler ultrason inceleme sonuçlarının prediktif özelliğini araştırmaktır.

Gereç ve Yöntemler: Bu çalışma, 2018-2020 yılları arasında ultrasonografi ve mamografi ile şüpheli lezyonları saptanan ve patolojik invaziv meme kanseri tanısı konan 47 hastanın üçüncü basamak bir hastanede dahil edildiği prospektif bir vaka kontrol çalışmasıdır. Aynı hastanın invaziv karsinom tanısı alan memede ve sağlıklı memede power doppler ultrasonografi ile LTA ve İTA ile değerlendirildi. Sağlıklı meme kontrol grubuydu.

Bulgular: Tanısal istatistiksel testlerde; LTA PSF değeri için optimum cut-off değeri 19.6 cm/s, bu değerin duyarlılığı %81.3 ve özgüllüğü %95.8'dir. LTA RI değeri için optimal cut-off değeri 0.805, bu değerin duyarlılığı %91.7 ve özgüllüğü %95.8 idi. Karşı memede invaziv meme kanseri tespiti için ITA PDF'nın 8.35'in altındaki duyarlılığı %77.1, özgüllüğü %79.2'dir.

Sonuç: Bildiğimiz kadarıyla, bu çalışma dahili ITA ve LTA'nın akış modellerini birlikte inceleyen ilk yayındır. Bu çalışma, meme kanserini tespit etmek için kullanılabilecek, kolay erişilebilir, uygulanabilir, yüksek duyarlılık ve özgüllüğe sahip yeni kantitatif tanı testleri sunmaktadır.

Anahtar Kelimeler: Meme tümörleri, Kan Akış Hızı, Doppler Ultrason Görüntüleme, Torasik Arterler

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Introduction

The most common malignancy in women is breast cancer and is the second most common cause of death after lung cancer worldwide [1]. Early diagnosis is the most important factor that increases survival in breast cancer, and the prognosis of the disease is directly related to the stage at the time of diagnosis [2]. Screening with mammography at a rate of 16-40% plays a role in the reduction of breast cancer deaths among women aged 40-74 [3,4]. However, it has been reported that mammography rates decrease as the breast density increases [5]. Screening by mammography alone may be insufficient in these women [6]

For the evaluation of breast lesions, breast ultrasonography is an important imaging method that complements mammography and has high sensitivity and specificity. It is easily possible to evaluate cystic lesions in the breast and to evaluate peripherally located breast lesions with breast ultrasonography [7-9]. However, the quality of ultrasound images for ultrasonographic evaluation is affected by many factors such as contrast and signal to noise ratio, acoustic shadowing and enhancement artifacts. In addition, it is subjective because it depends on the experience and skill levels of the person performing ultrasonography (10). Quantitative ultrasonographic parameters are investigated in order to decrease misdiagnosis in breast ultrasonography and to eliminate this subjective evaluation [11].

Internal thoracic artery (ITA), lateral thoracic artery (LTA) and internal mammarian artery (IMA) play a role in breast feeding. ITA nourishes the breast and anterior chest wall. IMA feeds the breast through the posterior and anterior medial branches and LTA feeds the lateral part of the chest [12,13]. Power doppler ultrasound (PDUS), on the other hand, has a diagnostic value in distinguishing benign and malignant masses of lesions by evaluating the blood flow and vascularization of solid lesions in gray scale ultrasonography (US) findings [14,15]. However, there are not enough studies investigating values such as resistive index (RI), pulsatility index (PI) of ITA and LTA in patients with malignant breast lesions.

The aim of this study is to investigate the predictive feature of LTA and ITA PDUS examination results of patients with malignant breast lesions.

Materials and Methods

Ethic Approval and Patients

This is a prospective case-control study in which 47 patients with suspicious lesions detected by ultrasonography and mammography and diagnosed with pathologically invasive breast carcinoma between 2018-2020 were included in the Adıyaman Training and Research Hospital. Local ethics committee approval was obtained for the study. The study was carried out in accordance with the Declaration of Helsinki, and a signed consent form was obtained from all participants or their legal guardians.

Being 18 years of age or younger, women diagnosed with malignancy at the time of diagnosis or previously, having a history of breast surgery in the last 12 months, receiving radiotherapy and chemotherapy in the last 12 months, infectious diseases such as periductal mastitis and granulomatous mastitis that have been treated in the last 12 months, having illnesses and being in the lactation period were exclusion criteria from the study.

Ultrasonographic examination

The evaluation of the patients was performed on a GE Logiq S8 (GE Healthcare, Milwaukee, WI, USA) ultrasound device using a 12-MHz linear probe.

Patients were evaluated primarily by US examination and B-Mode US examination by two radiologists with 5 years and 15 years of

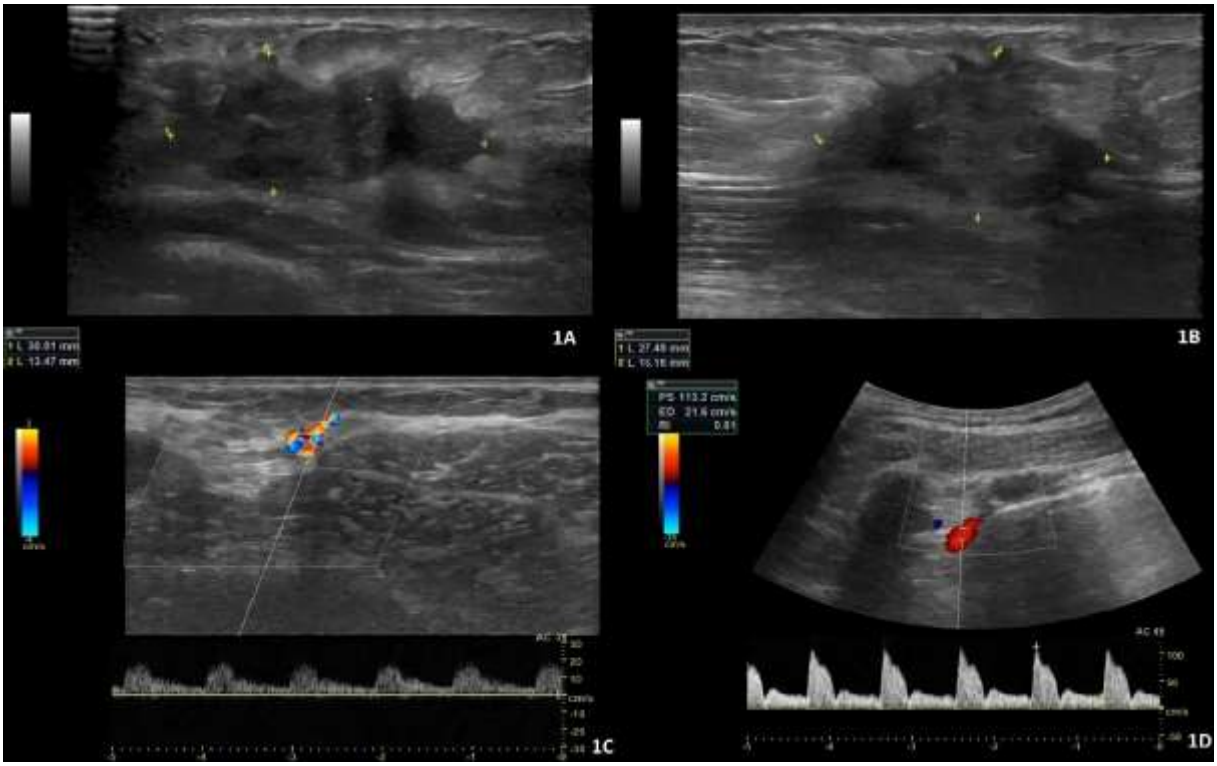

Figure 1:1A-1B An irregular shaped solid mass lesion in the upper outer quadrant of the right breast with a spicule contour, 30x27x15 mm in size, diagnosed as invasive ductal carcinoma. 1C:Evaluation of lateral thoracic artery by power doppler ultrasonography.1D: Evaluation of the internal thoracic artery by power doppler ultrasonography.

experience, and they were characterized using the Breast Imaging Reporting and Data System (BIRADS (American College of Radiology 2013)) classification system. The lesions in BIRADS categories 4 and 5 were lesions with a high probability of malignancy according to this classification. Location, size, lesion shape/margin features and echogenicity of the lesions were noted (Figure 1A-1B).

Biopsy Method

All lesions were sampled by US-guided core biopsy and pathological evaluation results were obtained for all lesions.

PDUS examination

Patients whose pathological examination results were presented as early-stage invasive breast carcinoma and who were decided to have an operation were performed an ultrasound examination before the operation. After the biopsy procedure, PDUS examination was performed 2-4 weeks later to prevent the flow parameter changes that may develop due to the procedure. PDUS imaging and spectral analysis were performed on the patient's ITA and LTA feeding the breast with lesions and the ITA and LTA feeding the healthy breast.

Patients were placed on their back with their hands under their heads and arms in flexion to detect LTA and ITA. The probe was first placed parallel to the edge of the pectoral muscle and LTA was detected with color doppler (Figure 1C). ITA was detected with color doppler by placing a probe in the second intercostal space of both sides (Figure 1D). ITA was located 1-2 cm lateral to the sternal border [16,17]. Spectrum examination in Doppler imaging was measured at the lowest pulse frequency repetition value that did not

Table 1. Patients demographic characteristics and lesion characteristics.

	Number	%
Age		
30-44	23	47,9
45-59	18	37,5
60 and above	7	14,6
Breast Pattern		
B	12	25,0
C	31	64,6
D	5	10,4
Breast Localization		
Right	19	39,6
Left	29	60,4
Lesion Localization		
Lower inner	10	20,8
Upper inner	8	16,7
Upper outer	15	31,3
Lower outer	7	14,6
Upper middle	8	16,7
Lesion Edge		
Spiculation	23	47,9
Angulation	11	22,9
Microlobulation	14	29,2
Lesion Shape		
Irregular	46	95,8
Oval	2	4,2

cause aliasing artifacts, the lowest Doppler inspection window, and a low wall filter (50 Hz). Peak systolic flow (PSF), peak diastolic flow (PDF), resistive index (RI) and Pulsatility index (PI) values were obtained for both arteries.

Statistical Analysis

Statistical analysis of all results was performed using the SPSS software version 22.0 (SPSS Inc., IBM Corp. Armonk, NY).

The Mann-Whitney U test was used for independent binary groups that did not fit the normal distribution. Categorical variables presented as ratio, continuous variables were presented as median (min-max) value and standart deviation (SD). A $p<0.05$ value was considered significant.

We used Reciever Operator Characteristics Curve (ROC) analysis to determine the effectiveness of the PDUS parameters and predict malignant breast lesions. Although the, LTA RI, LTA PSF, ITA PDF and ITA RI values were statistically significant in areas under the curve in the predict of malignant breast lesions and their specificities were high.

Results

The sociodemographic characteristics of the patients and the characteristics of the lesions are given in Table 1.

PSF, PDF, RI and PI values of LTA and ITA values were compared for the breast with the lesion diagnosed as invasive carcinoma and healthy breast.

LTA PSF, LTA RI and ITA RI values were found to be significantly higher in the invasive carcinoma side compared to the healthy side ($p<0.001$), and the ITA PDF value was significantly higher in the healthy side ($p<0.001$) (Table 2).

ROC Analysis Results

In deciding the diagnosis of invasive breast carcinoma, it was found that LTA PSF, LTA RI and ITA RI on the breast side diagnosed with carcinoma and ITA PDF values on the healthy breast side are very good diagnostic tests and the areas under the curve are significant. The values of the area under the curve (AUC) of these measurements are given in Table 3.

It has been determined that these values can be used to decide the distinction between invasive breast carcinoma and healthy breast. It was found that higher LTA PSF, LTA RI and ITA RI values in the breast with invasive breast carcinoma compared to the healthy

Table 2. Comparison of PDUS measurements of breast with malignant lesions and healthy breast*

	Control Breast			Invasive Carcinoma Detected Breast			
Measurement Value	Mean	S.D.	Median	Mean	S.D.	Median	p
LTA PSF	15,95	4,14	15,50	26,91	13,46	22,10	<0.001
LTA PDF	3,73	1,11	3,40	4,49	2,88	3,80	0.204
LTA RI	0,76	0,05	0,78	0,84	0,03	0,84	<0.001
LTA PI	2,03	0,35	2,11	2,11	0,18	2,15	0.323
ITA PSF	49,77	22,01	42,20	48,77	16,49	44,80	0.714
ITA PDF	10,68	3,77	9,40	6,95	3,59	6,40	<0.001
ITA RI	0,76	0,06	0,76	0,86	0,05	0,84	<0.001
ITA PI	3,10	0,57	3,22	2,99	0,55	3,22	0.336

LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow;
PDA: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index; SD: Standart Deviation
* Mann Whitney U

Table 3. ROC characteristics of various parameters

				% 95 Confidence Interval	
Variable	Area	Std. Error	p	Lower Bound	Upper Bound
LTA PSF	0,859	0,042	<0,001	0,777	0,942
LTA RI	0,944	0,027	<0,001	0,891	0,997
ITA PDF	0,784	0,048	<0,001	0,690	0,877
ITA RI	0,883	0,037	<0,001	0,811	0,956

LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow; PDA: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index

Table 4. Cut-off values and validity results

	Cut-off	Sensitivity	Spesifity	J statistic	LR+	LR-
LTA PSF	19,6	81,3	95,8	0,771	19,35	0,20
LTA RI	0,805	91,7	95,8	0,875	21,83	0,08
ITA PDF (low)	8,35	77,1	79,2	0,563	3,70	0,28
ITA RI	0,8	89,6	81,2	0,708	4,76	0,12

LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow; PDA: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index; LR: Likelihood ratio

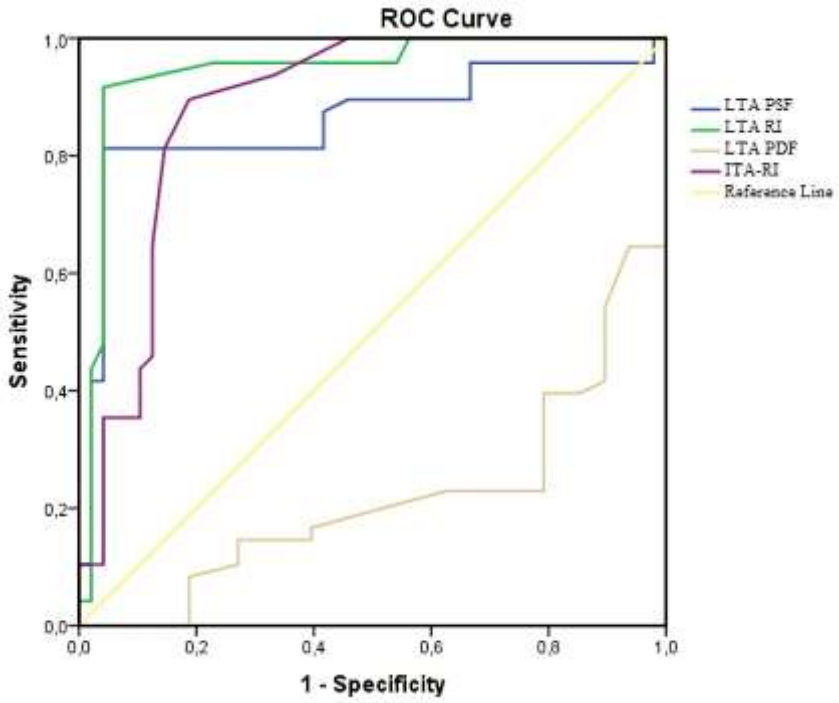

Figure 2: Area under curve in ROC analysis. LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow; PDF: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index

breast can predict the diagnosis of invasive breast carcinoma in the current breast. Low ITA PDF value in the healthy breast compared to the breast with invasive breast carcinoma can predict the diagnosis of invasive carcinoma in the other breast (Figure 2).

The optimal cut-off value for LTA PSF value is 19.6 cm/s, the sensitivity of this value is 81.3% and the specificity is 95.8%. If there are LTA PSF values above this cut-off value, that lesion can be interpreted in favor of a malignant lesion.

The optimal cut off value for LTA RI value was determined as 0.805, the sensitivity of this value was 91.7% and the specificity was 95.8%. The lesion detected at LTA RI values above this cut-off value can be interpreted in favor of invasive breast carcinoma (Table 4).

The sensitivity of the ITA PDF value below 8.35 for the detection of invasive breast carcinoma in the opposite breast is 77.1% and the specificity is 79.2%. Among these measurement values, the diagnostic value with the lowest false positivity and false negativity rates is the LTA RI value. The positive likelihood ratio value of this value is 21.83 and the negative likelihood ratio value is 0.008 (Table 4).

Discussion

According to the results of this study, from the quantitative data obtained in PDUS examinations of LTA and ITA arteries. The cut-off values of LTA PSA, RI and ITA RI values were determined to detect invasive breast carcinoma, and the sensitivity and specificity of these values are quite high.

Angiogenesis plays an important role in both local growth and distant metastases of breast cancer. Breast ultrasonography is an inexpensive diagnostic method that can be easily applied in young women with a family history, in patients admitted to outpatient clinics for reasons such as mastalgia, nipple discharge, and in women with palpable lesions on breast examination without radiation exposure [18]. In addition, it is more sensitive in detecting lesions that cannot be

detected in mammography in dense breasts [19].

The features of the masses detected in the breast such as their shape, contour features, and their location in the breast axis can be easily determined by ultrasonography. The characteristics of the masses can be determined by evaluating the vascularization of the masses with PDUS examination. In recent studies, it has been reported that PDUS examination is very important in increasing blood flow in the tumor and detecting neoangiogenesis and can be used to determine the malignant character of lesions [14,15].

It is known that new vascular structures that do not have a smooth muscle structure are observed among the malignant lesions detected in the breast [20]. Although it has been shown in various studies that the hypervascularity detected in US examination can provide information about the malignant character of the lesions. It has been stated that the RI and PI values detected in the lesions are not good diagnostic tools for lesion characterization [21]. In a recent study, it was shown that the diastolic flow reversed in the flow in the mass detected in the breast and the RI value above 1 was most likely associated with lesion malignancy [21]. However, it has been previously stated that lesion vascularization is not always evident in patients with breast cancer. Accordingly, PDUS evaluation can gain sensitivity when evaluated together with the morphological features of the mass identified in ultrasonography [22]. Although PDUS imaging is thought to be helpful in distinguishing benign and malignant solid masses, it has been stated that it does not have high predictive quantitative values. PDUS imaging can only be used to support the pre-diagnosis of lesions with suspicious morphological features in B-mode US examination [23].

The hypothesis of our study was that vascularization could change in the breast developing malignancy rather than lesion vascularization for malignancy characterization. Therefore, PDUS examination of the main vascular structures feeding the breast was performed and it was aimed to obtain quantitative data. In some previous studies investigating the value of LTA and axillary artery blood flow in determining the lesion characteristics in breast masses, have been conducted. It has been reported that a LTA RI value of 0.67 and above is significant for detecting malignancy in these studies [17,24]. However, even in the healthy breast determined as the control group in our study, LTA values were generally above 0.67. This explains why the sensitivity and specificity values of the values determined in our study are high. In addition, the statistical analyzes made in our study are more comprehensive and powerful. Moreover, as far as we know, there is no current publication where ITA evaluations were made, and in the results of our study, not only LTA RI but also LTA PSA and ITA RI values were obtained with high sensitivity and specificity predicting malignancy. This also supports our hypothesis. In addition, unlike other studies, this study allows for easily calculable evaluations rather than spectral examinations such as negative diastolic flow, which are rare and not always possible to detect.

According to these data, LTA RI, LTA PSF, ITA PDF and ITA RI values may be a new diagnostic method with high sensitivity and specificity that can be used for the detection of invasive breast carcinoma.

The strength of this study is that it is prospective. Another strong feature is that multiple parameters belonging to LTA and ITA arteries work together with their diagnostic features and obtain cut-off values with high sensitivity and specificity values.

This study had some limitations. First of all, the study population was small, because the patients included in this study consisted of

patients who were made surgical preparations immediately after their diagnosis, and patients diagnosed with locally advanced breast cancer who were diagnosed with neoadjuvant chemotherapy in our center were excluded. It was thought that the application of neo-adjuvant chemotherapy would affect breast vascularization, and measurements were not taken from these patients before the operation. Second, the evaluations of the patients were made only for lesions diagnosed with pathologically invasive breast carcinoma. Further studies with larger populations including vascular changes of benign lesions can be conducted.

Conclusion:

In conclusion, this study presents new quantitative diagnostic tests with high sensitivity and specificity, easily accessible, and applicable in detecting breast cancer.

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Original Article

Frequency of Incidental Pulmonary Findings Detected on PET/CT Images of Elderly Patients Diagnosed with Extrapulmonary Malignant Neoplasm

Ekstrapulmoner Malign Neoplazm Tanılı Yaşlı Hastaların PET/BT Görüntülerinde İnsidental Pulmoner Bulguların Sıklığı

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ABSTRACT

Introduction: Reporting thorax imaging findings on 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET/CT) is important for patient management. Even if some pulmonary findings are benign, they can have serious life-threatening consequences. This study aimed to investigate the frequency of benign or malignant pulmonary findings, which were simultaneously detected in PET/CT scans, of elderly patients with extrapulmonary malignant neoplasms.

Methods: Patients aged ≥65 years, applying to nuclear medicine department of a tertiary level health unit between November 2017 and April 2018 were retrospectively evaluated. Demographic and clinical information and PET/CT scans were obtained from their previous hospital records. Data obtained were analyzed using the SPSS version 22.

Results: A total of 112 patients (mean age, 72.8 ± 7.0; females, 58.9%) were included in the study. In total, 38.4% of the patients had a smoking history, and 39.2% were exposed to second-hand smoke. The most common indications for PET/CT imaging were post-treatment evaluation (42.9%) and staging (35.7%). Predominantly diagnosed malignancies were cancers of the gastrointestinal system (26.8%), breast (26.8%), and urogenital system (17%). While most patients had benign or malign pulmonary findings in thoracic images, no abnormal pulmonary findings were observed in only 24 patients (21.4%). The most common findings were emphysema (39.3%), metastatic nodules (27.7%), bronchial wall calcifications (14.3%), and air trapping/cysts (9.8%).

Discussion and Conclusion: This study revealed that 78.6% of elderly patients with extrapulmonary malignant neoplasms undergoing PET/CT scans had at least one pathologic lung finding. Although most of these findings are benign, reporting of them is important in the management and clinical outcomes of patients with malignancy.

Keywords: Elderly patient, PET/CT, malignant neoplasm, incidental pulmoner findings

ÖZET

Giriş ve Amaç: 18F-fluorodeoksiglukoz (18F-FDG) pozitron emisyon tomografi-bilgisayarlı tomografide (PET/BT) malignite kuşkulu lezyonlara ilaveten, benign karakterdeki bulguların raporlanması da hasta yönetiminde önemlidir. Bazı pulmoner bulgular benign olsa bile hayati tehlike oluşturan ciddi sonuçlara yol açabilir. Bu çalışmanın amacı, ekstrapulmoner malign neoplazm tanısı ile PET/BT çekilmiş olan yaşlı hastalarda benign ya da malign pulmoner bulguların sıklığını araştırmaktır.

Yöntem ve Gereçler: Üçüncü basamak sağlık kuruluşu nükleer tıp bölümüne Kasım 2017-Nisan 2018 tarihleri arasında başvuran 65 yaş ve üzeri hastalar retrospektif değerlendirildi. Demografik verileri, klinik bilgileri ve PET/BT görüntüleri hastane kayıtlarından elde edildi. Bulgular SPSS 22 kullanılarak analiz edildi.

Bulgular: Toplam 112 hasta (yaş ortalaması 72.8±7.0; %58.9'u kadın olan) çalışmaya dahil edildi. Hastaların %38.4'ü aktif, %39.2'si pasif sigara içicisiydi. En sık PET/BT çekimi endikasyonu, tedavi sonrası yanıt değerlendirme (%42.9) ve evreleme (%35.7) idi. Tanıların çoğunluğunu gastrointestinal

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sistem (%26.8), meme (%26.8) ve ürogenital sistem (%17) maligniteleri oluşturmaktaydı. Hastaların çoğunda toraks kesitlerinde benign ya da malign pulmoner bulgulara rastlanırken, sadece 24 hastada (%21.4) hiçbir anormal pulmoner bulgu izlenmedi. En sık saptanan bulgular; amfizem (%39.3), metastatik nodül (%27.7), bronş duvarı kalsifikasyonu (%14.3) ve hava hapsi/kisti (%9.8) idi.
Tartışma ve Sonuç: Bu çalışma, ekstrapulmoner malign neoplazm tanısı ile PET/BT çekilen yaşlı hastaların %78.6'sında en az bir anormal pulmoner bulgunun olduğunu gösterdi. Bu bulguların çoğu iyi huylu olmakla birlikte, bunların raporlanması malignitesi olan hastaların yönetimi ve klinik gidişatında önemlidir.

Anahtar Kelimeler: Yaşlı hasta, PET/BT, malign neoplazm, insidental akciğer bulguları

Introduction

18F-fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET/CT) is a valuable imaging modality commonly for early detection, accurate staging, and evaluating treatment response of many cancers. Its main advantage in relation to other radiological imaging methods is the availability of functional imaging [1]. Oncologic PET/CT scans can be used to view cross-sectional tomographic and functional images from the vertex to the proximal thigh. Besides its ability to detect malignancies, PET/CT also reveals non-malignant findings.

The incidence of malignant diseases is increasing due to several factors such as advanced age, increased smoking rate, genetic factors, poor nutrition, air pollution, and occupational exposure. Imaging techniques exert an important role in the management of patients with malignant diseases. Among them, PET/CT has gained relevance not only for diagnosis but also for staging and treatment outcome evaluation. The rate of incidental findings has increased along with the use of low dose CT, and the importance of non-malignant incidental findings for patient management is well established among clinicians [2]. Therefore, during clinical evaluation it is crucial to carefully examine non-diagnostic CT images in the oncologic PET/CT scans [3]. It is recommended that such additional findings should be taken into account, as they are important for disease management and prognosis [4].

In the elderly patients, it is crucial to distinguish between age-related and pathological findings to avoid misdiagnoses [5]. Therefore, special knowledge on diagnostic imaging of elderly patients is required [6].

The aging causes reduced lung elasticity due to the loss of supportive tissue. Homogeneous airway dilatation may occur in the absence of inflammation, fibrosis, alveolar wall destruction or distortion [7,8]. Emphysematous and fibrotic changes in the basal segments of lung parenchyma are commonly seen, as well as morphological changes such as progressive calcifications of the airways and thorax [7-9].

A previous study reported that the CT images of elderly (> 65 years) showed a significant number of asymptomatic emphysema than those of young adults (<55 years). The same study found that 60% of elderly adults exhibited interstitial changes of the subpleural reticular pattern [10]. Another study reported that asymptomatic air cysts were frequently observed in elderly patients [11]. A study showed that asymptomatic older patients had greater prevalence of air trapping and extensive degree of air trapping also correlated with aging [12].

The aim of the present study was to evaluate pulmonary findings of elderly patients undergoing PET/CT scans due to extra-pulmonary malignant neoplasms. The results will also provide an overview on the non-

malignant uses of PET/CT in elderly population.

Materials and Method

Setting and participants

The PET/CT scan of cancer patients sent to the department of nuclear medicine at a tertiary care unit in Turkey was retrospectively analyzed. The institutional ethical committee approved this study.

Demographic (i.e., age, gender, smoking status, comorbidities) and clinical (i.e., diagnosis, PET/CT indications) information were obtained from the hospital's database and the PET records/CT informed consent forms. Patients aged <65 years and diagnosed with lung cancer were excluded from the study.

The study was approved by the Recep Tayyip Erdoğan University of Local Ethics Committee (protocol number: 2018/109), and Helsinki declaration principles were followed.

18F-FDG PET/CT scan

All patients fasted for at least 6 hours before PET/CT scans. For all patients, information about the PET/CT scanning indication, clinical history, chemotherapy and radio-therapy history, height and weight, and fasting blood glucose levels were recorded before 18F-FDG injection. All patients had a fasting and blood glucose level was <200 mg/dL before imaging. The approximately 220–370 MBq 18F-FDG was intravenously administered to each patient. Following a resting period of approximately 50–60 min in the waiting room, the patients were taken for the PET/CT scan (by Siemens Biograph mCT, 20 excel). Images were acquired from the vertex to the upper thigh. CT images were taken with a 5 mm slice thickness and an average of 120-kVp/100-mAs dose range without intravenous contrast. PET images were acquired within 2–4 min per bed. Both the PET and CT images were obtained during normal tidal breathing.PET images were reconstructed using CT for attenuation correction. PET, CT, and fused PET/CT images displayed as coronal, sagittal, and transaxial planes were viewed on a syngo-via workstation (Siemens Healthineers). A nuclear medicine physician interpreted all images. All incidental lung findings detected on the PET/CT images were reported.

Statistical analysis

All statistical analyses were performed using the SPSS version 22. Descriptive statistical methods were used for demographic, clinical, and radiological features. Categorical variables were analyzed using the Pearson's Chi-square test, whereas continuous variables were analyzed using the student's t-test. A level of $p<0.05$ was considered statistically significant.

Results

A total of 112 patients were evaluated. The mean age was 72.8±7 and 58.9% were females. The proportion of current and past smokers was 38.4%, and 24% reported second-hand smoke exposure. A total of 76.8% of the patients had at least one comorbid disease, including hypertension (65.2%), diabetes mellitus (14.3%), and coronary artery disease (11.6%). The patients' demographic data is presented in Table 1.

The patients were referred from the medical oncology (51.8%), general surgery (13.4%), hematology (12.5%), radiation oncology (9.8%) departments respectively for PET/CT scan.

Most patients undergoing PET/CT scan had gastrointestinal malignancies (26.8%), breast cancer (26.8%), and urogenital malignancies (17%). The proportion of patients with chemotherapy and radiotherapy histories was 63.4%and 25.8%, respectively. PET/CT scan indications included staging (35.7%), post-

Table 1. Demographic characteristics of all patients

Variable	n (%)
Age (mean, ±SD)	72.8 ± 7
Gender	
Female	66 (58.9)
Male	46 (41.2)
Smoking status	
Current/former smoker	43 (38.4)
Presence of second hand smoke exposure	27 (24.1)
Presence of comorbid disease	86 (76.8)
Presence of obstructive lung disease	21 (18.8)
Presence of inhaler medication use	22 (19.6)
Presence of tuberculosis or pneumonia history	11 (9.8)

Table 2. Pulmonary findings of the study population

Pulmonary findings	n (%)
Emphysema	44 (39.3)
Metastasis	31 (27.7)
Bronchial wall calcification	16 (14.3)
Air trapping/Cyst	11 (9.8)
Calcific nodule	10 (8.9)
Sequela fibrotic changes in apex	10 (8.9)
Bilateral pleural effusion	4 (3.6)
Reticular/Reticulonodular infiltration	6 (5.4)
Pleural thickening	5 (4.5)
Solitary pulmonary nodule	3 (2.7)
Calcific pleural thickening	2 (1.8)
Subsegment atelectasis	3 (2.7)
Bronchiectasis	1 (0.9)
Collapse and consolidation	1 (0.9)
Ground glass opacity	1 (0.9)

treatment evaluation (42.9%), and re-staging (21.4%) respectively.

Pathologic lung imaging findings were observed in 78.6% of the patients. The most common findings were emphysema (39.3%), metastatic nodular lesion (27.7%), and bronchial wall calcification (14.3%). Emphysema and metastatic lung nodules are shown in Figure 1, 2 and collapse-consolidation image determined by PET/CT for evaluation of treatment response after chemotherapy is given in Figure 3.

Table 3. Comparison of the variables according to patients' smoking status

	Current/former smoker (n:43)	Non-smoker (n:69)	p
Gender male	38 (88.4%)	8 (11.6%)	<0.001
Presence of obstructive pulmonary disease*	9 (20.9%)	12 (17.4%)	>0.05
Incidental pulmonary findings	41 (95.3%)	47 (68.1%)	<0.001
History of tuberculosis and pneumonia	5 (11.6%)	6 (8.7%)	>0.05

*Asthma+ COPD patients

Information about pulmonary findings is presented in Table 2.

A total of 43 patients (38.4%) had current/ former smoking history and most were male (88.4%). Most of the smokers (95.3%) had pathologic lung findings (p <0.005). Table 3 shows the patient characteristics according to their smoking status.

Among the 112 patients, 21 of them were diagnosed with obstructive pulmonary disease, 11 patients had chronic obstructive pulmonary disease (COPD) (52.4%), and 10 patients had asthma (47.6%). The rate of current/former smokers was 20.9% of the total but it was 72.8% among patients with COPD.

Lung metastases were most commonly caused by breast (29%) and colon cancers (22%). Other cancers associated with lung metastasis were bladder cancer (6%), renal cell carcinoma (6%), endometrium carcinoma (6%), cancers of undifferentiated origin (6%), gastric cancer (3%), larynx cancer (3%), pancreatic cancer (3%), liver cancer (3%), sarcoma (3%), and multiple myeloma (3%) respectively.

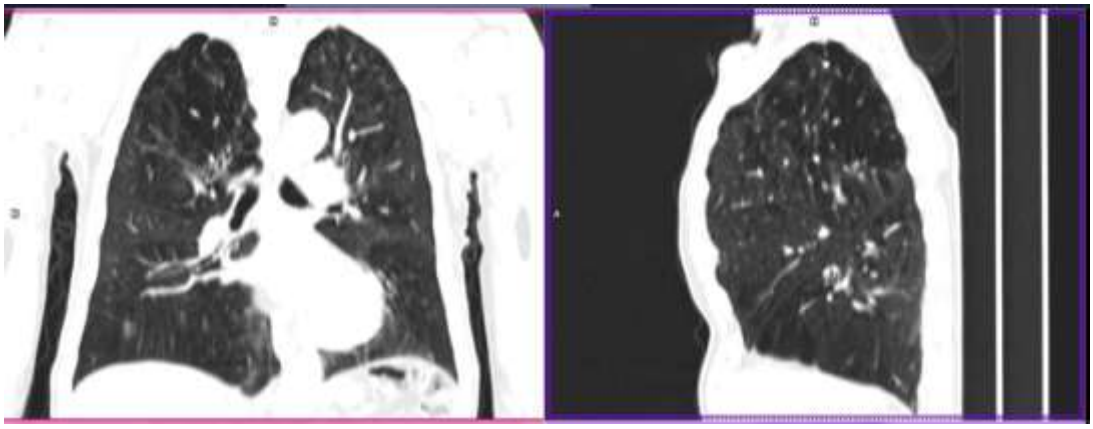

Figure 1. Emphysematous lung image in coronal and sagittal sections on CT

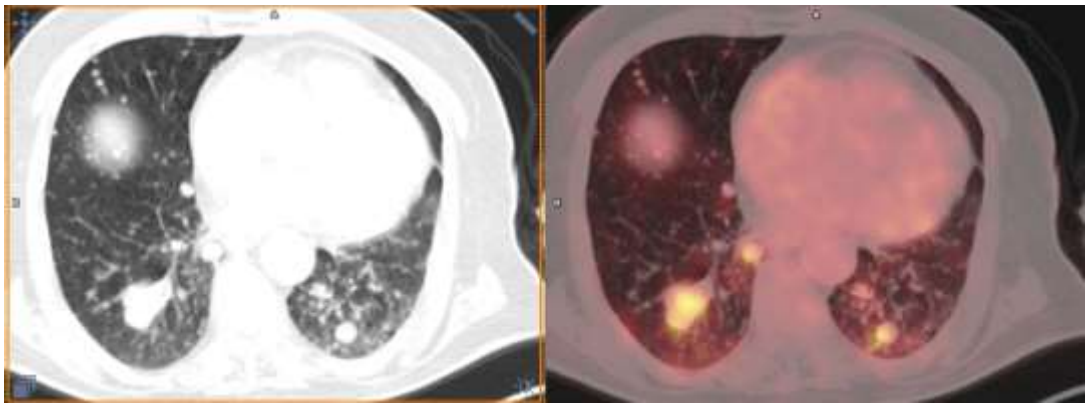

Figure 2. Transaxial CT (a) and fusion image (b) on PET/CT scan showed multiple lung metastases in a 64-year-old male patient diagnosed with a rectal cancer

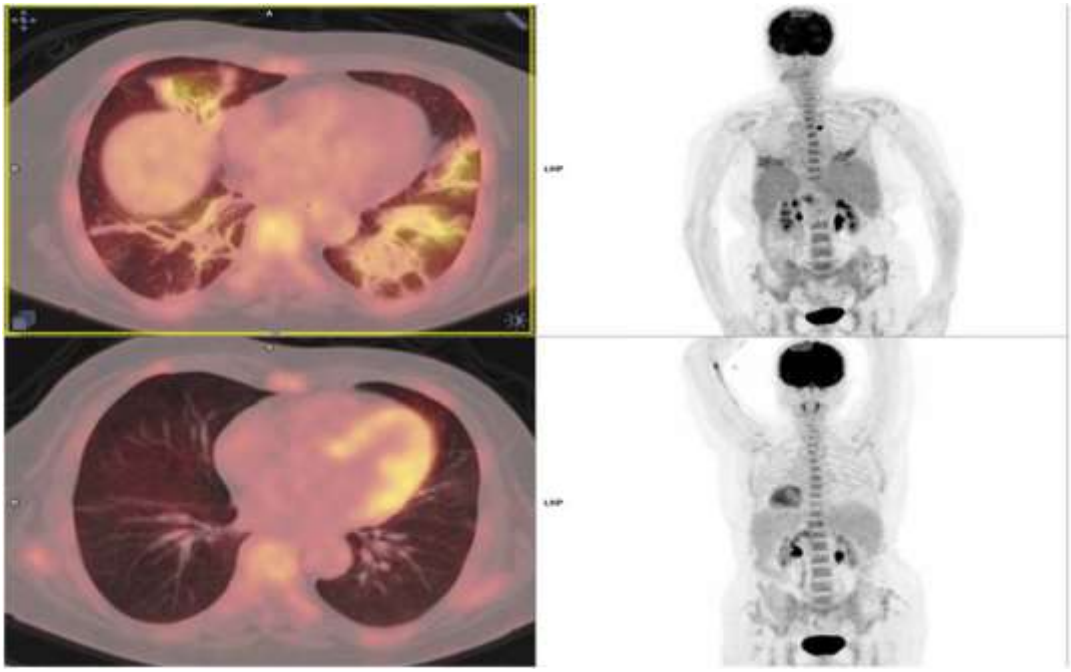

Figure 3. Transaxial fusion PET/CT (a, b) and maximum intensity projection (c, d) images showed post-chemotherapy collapse and consolidation (a, c) in a 77-year-old male patient diagnosed with a hematologic malignancy

Discussion

In the present study, thoracic CT images of 112 elderly patients undergoing PET/CT scans were retrospectively examined. Among all patients, only 24 of them (21.4%) had no pathologic lung findings on thoracic CT images. The most common pathologic findings were emphysema (39.3%), metastatic lung nodules (27.7%), bronchial wall calcifications (14.3%), and air trapping/air cysts (9.8%). Other findings included calcific nodules, sequelae fibrotic changes in the apex, subpleural reticular/ reticulonodular infiltration, and solitary pulmonary nodules. Calcific pleural thickening, subsegmental atelectasis, bronchiectasis, collapse/ consolidation, and ground glass opacity were less commonly found. All these findings significantly more frequent in the patients with a smoking history ($p < 0.001$).

It is known that with aging, distal airspaces expand due to the loss of supportive tissue, resulting in changes designated as "senile lung, senile hyperinflation, or senile emphysema". Homogeneous airway dilatations can be observed in elderly patients in the absence of inflammation, fibrosis or other structural pathologies [7]. A study comparing the radiological findings in the lung parenchyma of elderly (>75 years) and younger individuals (<55 years), found a higher rate of centrilobular emphysematous changes in the elderly group. The same study found a 60% rate of interstitial changes with subpleural reticular pattern among elderly individuals [10]. Another study showed that 25% of asymptomatic elderly patients presented small cystic lesions [11]. In the present study, the most common incidental lung finding was emphysema, which is consistent with that observed in the existing literature (39.3%). However, the rate of interstitial changes associated with the subpleural reticular pattern was only 4.5%, which may have been due to the CT image technique used. In the present study, CT images were acquired at an average of 120-kVp and 100-mAs dose, in 4-mm section thickness and in inspiratory or expiratory phases instead of 120-kVp and 400-mAs dose, 1-mm section thickness, thin-section CT. This may have caused the inconsistency between the results of the present study and those of the existing literature [11,12]. In the present study, the rate of air cysts was 9.8%, whereas previous studies report a rate of 25% [11]. This discrepancy may have also been caused by the used image section thickness.

Lung is one of the most common sites for primary and metastatic malignancies and a challenging site to diagnose primary versus a metastatic origin of the tumor on cytology. The developments in CT technology have led to an increasing detection of pulmonary nodules in thorax CT. A previous study reported that 20% of the patients with extra-pulmonary malignant neoplasm presented metastatic lung nodules on thorax CT images [13]. In the latter study, thorax images were evaluated using thin-section CT (section thickness, 1 mm). The detection of incidental pulmonary nodules has increased with the use of thin-section thorax CT [14]. In our study, metastatic lung nodules were detected in 27.7% of the cases, although images were not acquired using thin-section CT (section thickness, 4 mm). This ratio is relatively high compared with that reported in the literature. In our department, PET/CT images have a 5-mm thickness and thus have low sensitivity for nodules <4 mm. Therefore, there is a possibility for small nodules to go undetected. However, histopathological sampling of the detected lung nodules was not performed. The presence of multiple nodules on PET/CT images and FDG uptake levels of nodules are findings that support the occurrence of metastases. Since most available nodules present these features, they have been

clinically accepted as metastases. The clinical history of a known extra-pulmonary primary and the radiologic findings of multiple nodules in the lung are useful in arriving at the right diagnosis but is not always reliable. The approach to the diagnosis of metastatic tumors in the lung on cytology should be largely guided by the previous clinical history and comparison with previous tissue/cellular material if available [15]. Some nodules classified as malignant may, in fact, be benign, which helps to explain our findings of a higher rate of lung metastases compared with those reported by previous studies.

When the high occurrence of lung metastasis is analyzed from a different perspective, it becomes questionable whether cancer diagnosis is delayed in elderly patients. In the previous literature, 20% of lung metastases were detected without discriminating between old and young patients [13]. Pulmonary metastases are hematogenous distant metastases. In the present study, a higher rate of metastatic lung nodules than that reported in the previous literature was found, which may be related to the fact that elderly patients are diagnosed with advanced stage cancers. Comorbidities in elderly patients may overshadow cancer-related symptoms, thereby delaying cancer diagnosis. Therefore, a careful evaluation of geriatric patients should be performed, excluding malignancies in the differential diagnosis.

According to the previous literature, lung metastases were most commonly caused by breast cancer, followed by colon cancer [16]. Accordingly, in the present study the most common causes of lung metastasis were breast (29%) and colon cancers (22%).

Among the patients analyzed in the present study, only 21.4% had normal thorax CT images. Most patients had one or more pathological findings. Beside the metastatic malignant lesions, reporting other incidental lung findings such as emphysema, collapse/ consolidation, bronchiectasis, pleural effusion, and interstitial pattern can contribute to improve the patients' quality of life through administration of adequate treatment and interventions. Such findings are therefore important for the subsequent management of cancer patients.

Conclusion

The prevalence of the geriatric patient population is increasing nationally and worldwide, leading to a higher cancer incidence. The present study investigated thorax images obtained from PET/CT scans of the geriatric patient population with extrapulmonary malignant neoplasms. Most patients (78.6%) had one or more pathologic lung findings. Consistent with the previous literature, our most frequent finding was emphysema. We found lung metastasis as the second most common finding, which contradicts previous studies. Although the interstitial changes are reported with a high rate in the existing literature, our study found a low rate, which was considered to be caused by the imaging procedures' differences.

CT scans of the lung parenchyma show common findings in elderly patients, which are thought to be related with collagen changes. The differential diagnosis between aging-related imaging findings and those secondary to disease is difficult and can not be achieved using imaging studies alone. These lesions should be compared with previous examinations and follow-up [7,10,17-19]. Therefore, the reporting of incidental findings in the geriatric population diagnosed with extrapulmonary malignancy may contribute to improve patient management.

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Original Article

The Predictive Value of FDG-PET / CT in Assessing Bone Marrow Involvement in Hodgkin Lymphoma Patients; A Single Center Experience

Hodgkin Lenfoma Hastalarında Kemik İliği Tutulumunu Değerlendirmede FDG-PET/BT'nin Prediktif Değeri: Tek Merkez Deneyimi

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ABSTRACT

Purpose: This study was conducted to determine the predictive value of positron emission tomography (PET/CT) used in staging Hodgkin Lymphoma (HL) at the time of diagnosis in determining bone marrow (BM) involvement.

Material and method: The patients diagnosed with Hodgkin lymphoma in our hematology department between 2009-2019 were analyzed retrospectively. The study included a total of 46 patients who underwent both BM biopsy and PET/CT for staging at the time of diagnosis.

Results: The mean age of the 46 patients was 40 years (19-80). BM involvement was determined in three patients from BM biopsy and in 14 patients from PET/CT performed at the time of diagnosis. When PET/CT results were analyzed according to BM biopsy results, it was found that the sensitivity was 100% (3/3) and the specificity was 74.4% (32/43).

Conclusion: The sensitivity of PET/CT is very high in detecting BM involvement in HL patients, and is a non-invasive test. However, in doubtful cases it may be more appropriate to perform a BM biopsy, even though it is invasive, as PET/CT may cause false positive patient staging due to its low specificity.

Keywords: Hodgkin lymphoma, bone marrow involvement, PET/CT

ÖZET

Giriş ve Amaç: Hodgkin Lenfoma hastalarında tanı anında evrelemede kullanılan PET/BT tetkikinin kemik iliği tutulumunu belirlemedeki yerini saptamak

Yöntem ve Gereçler: Sağlık Bilimleri Üniversitesi Ankara Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi Hematoloji kliniğinde 2009-2019 yılları arasında takip edilen Hodgkin lenfoma tanılı hastaların dosyaları retrospektif olarak incelendi. Bu hastalar arasında tanı anında hem kemik iliği biyopsisi yapılan, hem de PET/BT çekilen toplam 46 hasta çalışmaya dahil edildi.

Bulgular: Toplam 46 hastanın yaş ortalaması 40 (19-80) idi. Hastaların kemik iliği tutulum durumları incelendiğinde kemik iliği biyopsisinde üç hastada tutulum saptanırken, tanı anında yapılan PET/BT'de 14 hastada tutulum olduğu görülmüştür. PET/BT sonuçlarının kemik iliği biyopsi sonuçlarına göre değerlendirildiğinde duyarlılığının %100 (3/3), özgüllüğünün ise %74,4 (32/43) olduğu görüldü.

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Tartışma ve Sonuç: Hodgkin lenfoma hastalarında kemik iliği tutulumunu saptamada non invaziv bir test olan PET/BT'nin duyarlılığı çok yüksektir. Ancak özgüllüğünün düşük olması sebebi ile yanlış pozitif hasta evrelemesine sebep olabileceğinden şüphe halinde invaziv de olsa kemik iliği biyopsisi yapılması daha uygun olacaktır.

Anahtar Kelimeler: Hodgkin Lenfoma, kemik iliği tutulumu, PET/BT

Introduction

Hodgkin lymphoma (HL) is a lymphoid malignancy originating from B cells [1], which accounts for approximately 12% of all lymphomas [2]. In histomorphological classification it is divided into two major groups; classical type and nodular lymphocyte predominant (NLP) type. Classical type HL includes nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich (LR) and lymphocyte depleted (LD) subtypes [1].

Approximately 90% of cases are classical type HL patients. NS type is the most common subtype of classical HL. Although the average age at diagnosis varies between 20 and 34 years, differences can be detected between ethnic groups. It is generally seen more frequently in males than females. As with most other hematological malignancies, one of the most important prognostic markers in HL patients is stage. Correct staging of patients at the time of diagnosis plays a major role in the future management of patients. The Ann-Arbor classification with Cotswold modification is still used for staging HL [3].

With Cotswold modification, liver biopsy and laparotomy are removed from the routine staging of HL patients, while another invasive procedure, bone marrow (BM) biopsy, is still performed for staging [4]. Stage I-II is defined as early stage and stage III-IV as advanced stage disease [5]. Even if BM involvement exists alone, it advances the stage and therefore has significant impact on the treatment and prognosis [6].

BM involvement is an indicator of generalized disease since these cases are regarded as stage IV regardless of lymph node involvement. Although BM biopsy has been accepted as the gold standard to detect the involvement of BM since the 1970s, it is an invasive procedure and can cause some serious complications [4].

However, fluorodeoxyglucose (FDG) PET/CT has recently been the subject of research on the topic of determining BM involvement [6]. In the last 20 years, FDG / PET-CT has been used extensively for staging lymphomas. Several studies have retro-spectively investigated the use of PET-CT to detect BM involvement, and have reported that FDG/PET-CT showed superior sensitivity compared to BM biopsy. Of these studies, Tzu-Hua et al[7] showed the sensitivity of PET / CT to be 96.8%, whereas the sensitivity of BM biopsy was 32.3%. In another study, Weiler-Sagie et al[8] reported PET-CT sensitivity as 97%, and the sensitivity of BM biopsy as 15%. According to the results of the German Hodgkin working group, PET-CT performed at the time of diagnosis was reported to describe the involvement of BM with high sensitivity proved by BM biopsy. With excellent negative predictive value, PET is a very accurate and reliable tool for excluding BM involvement. [4].

The aim of this study was to determine the role of PET/CT examination used in staging at the time of diagnosis in determining BM involvement in HL patients diagnosed in our center.

Patients and methods

A retrospective evaluation was made of patients with HL diagnosed in the Hematology

Table 1. Demographic and clinical features of patients

Variable (N=46)		(n, %)
Disease subtype		
	LR	4 (8.7)
	MC	11 (23.9)
	NLP	7 (15.2)
	NS	24 (52.2)
Age at diagnosis (median, years)		40.0 [19.0-80.0]
Gender		
	Male	25 (54.3)
	Female	21 (45.7)
Stage at diagnosis		
	I	2 (4.3)
	II	26 (56.6)
	III	14 (30.4)
	IV	4 (8.7)

LR: Lymphocyte rich, MC:Mixed cellularity, NLP:Nodular lymphocyte predominant, NS:Nodular sclerosis

Clinic of the University of Health Sciences Ankara Diskapı Yildirim Beyazit Training and Research Hospital in the period 2009-2019. Of these patients, 46 who underwent both BM biopsy and PET/CT at the time of diagnosis were included in the study. Patients who had only BM biopsy or PET-CT at the time of diagnosis were excluded from the study. Age, gender, HL type and subtype, and disease stage were recorded. BM biopsy was considered the gold standard for the detection of involvement.

Statistical analysis

Study data were analyzed using SPSS 24 software. Descriptive statistics and frequency tables were used. Descriptive data were given as a percentage. Negative predictive and positive predictive values, sensitivity and specificity values were calculated.

Ethical approval and informed consent

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. As a standard of care/action of our hospital, the patient records confirmed that all the study patients gave informed consent at the time of hospitalization and before the administration of any intervention. The study was approved by University of Health Sciences Turkey, Ankara Dışkapı Yıldırım Beyazit Training and Research Hospital Ethics Committee (protocol no: 90/05, date: 22.06.2020).

Results

Of the 46 HL patients included in the study, 25 (54.3%) were male and 21 (45.7%) were female. The median age at diagnosis was 40.0 years (19.0-80.0). 84.8% of the patients were classical type HL and 15.2% were NLP type HL. The most common subtype in patients with classical type HL was NS in 24 (52.2%) patients. Other subtypes were MC in 11 (23.9%) patients and LR in four (8.7%) patients. No patient with LD subtype was detected. At the time of diagnosis, 28 (60.9%) patients were early stage (stage I-II), and 18 (39.1%) were advanced stage (stage III-IV). The demographic and clinical features of the patients are given in Table 1. When the BM involvement status of the patients was examined, involvement was detected in three patients in BM biopsy, and PET/CT showed increased uptake of FDG in 14 patients, demonstrating involvement at the time of diagnosis. BM involvement rates according to the diagnostic methods are given in Table 2. As a result of the analyses made by comparing the results according to the results of BM biopsy, the sensitivity of the PET / CT method in terms of BM involvement was 100% (3/3) and specificity was 74.4% (32/43) (Table 3).

Discussion

All HL patients should be staged during diagnosis to determine the appropriate treatment protocol and prognosis. History, physical examination, radiological imaging, and BM biopsy are part of the routine staging

Table 2. Bone marrow involvement rates according to diagnostic methods

(N=46)		Bone marrow involvement in bone marrow biopsy		Total
		(+) (n)	(-) (n)	
Bone marrow involvement in PET/CT	(+) (n)	3	11	14
	(-) (n)	0	32	32
Total		3	43	46

Table 3. PET/CT sensitivity, specificity and positive-negative predictive values

	Value	%95 confidence interval
Sensitivity	%100	29.24-100
Spesificity	%74.42	58.83–86.48
Positive predictive value	%21.43	14.08–31.23
Negative predictive value	%100	

process [9, 10]. Correct staging is important to be able to determine the appropriate treatment protocol. In addition, it is important in terms of side-effects and reducing toxicity [11]. BM involvement in HL patients is compatible with stage 4 disease. Although BM involvement rates vary according to the developmental level of countries, it is seen in approximately 10% of the adult population. [9, 10] In the current study, BM involvement was observed in three patients (6.5%) according to the histopathological findings.

BM biopsy is an invasive procedure used to diagnose a large number of hematological diseases and to evaluate treatment response. The posterior iliac crest is considered the most suitable region for biopsy. The frequency of complications is very low during and after this procedure (0.05%). The most common complication is bleeding.[12] Other complications can be listed as local infection at the biopsy site and needle breakage during the procedure. More rarely, transient neuropathy accompanied by gluteal compartment syndrome secondary to bleeding at the biopsy site may occur. In addition, patients with osteoporosis or osteomyelitis are at risk of bone fracture at the biopsy site. [12-14]

Since the procedure is invasive and there is a slight risk of the complications mentioned above, other methods that can be used instead of this procedure have been the subject of research. The first of these is the PET/CT method, which is currently used very often for staging. PET/CT is widely used in the initial staging of patients diagnosed with lymphoma and in evaluating the response to treatment. [11] In addition, it has high sensitivity and specificity in detecting extranodal involvement.[6] In this study, in which it was

aimed to determine the potential role of PET CT in detecting BM involvement at the time of diagnosis in HL patients, the negative predictive value and sensitivity of PET-CT was 100% and specificity was 74%. Eser A. et al.[15] analysed 104 patients with HL and the negative predictive value and sensitivity of PET-CT was reported to be 100% and specificity was 68.9%. In a study by Muzahir S. et al. [11] 122 patients diagnosed with HL were evaluated. The sensitivity of PET-CT was reported as 100%, specificity as 76.5% and the negative predictive value was 76.5% in terms of BM involvement.

Considering the data of the current study and other data in the literature, it can be seen that the sensitivity of PET / CT is quite high but the specificity is relatively low. The reason for this can be interpreted in several aspects. In PET-CT examination, 18F-FDG (a glucose analog) is taken into the cell with glucose transporter protein. Malignant cells have higher metabolic rates and express a greater number of specific membrane proteins than normal cells. As a result, 18F-FDG is taken up more by tumor cells. This is the basis of the FDG-PET imaging method. [16] As PET-CT is primarily a glucose measurement, it can give false positive results in cases where there is increased BM activity. Increased inflammatory markers, and inflammatory changes in the BM are examples of these conditions. Diffuse involvement may be associated with myeloid activation, which may also help to explain the low specificity of PET-CT in detecting BM involvement. [17] In addition, increased involvement in the sacral region may be observed in patients with B symptoms due to increased cytokine production. [2] Although the possibility is low, if PET/CT imaging were to be performed after BM biopsy, there could be a false increased involvement in the biopsy site.[18]

The limitations of this study are that it was performed retrospectively and with a limited number of patients. There is a need for further prospective studies with more patients.

Conclusion

The PET/CT method has high sensitivity in detecting the BM involvement of HL patients. Therefore, in patients with no BM involvement on PET-CT, there can be considered to be no need for the invasive and painful procedure of biopsy. However, since it may cause false positive staging due to low specificity, it will be more appropriate to perform BM biopsy in cases of high suspicion even though it is an invasive procedure.

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Original Article

Potential Novel Prognostic Factors in Malign Mesothelioma: Systemic Inflammatory Indices (SII) & Albumin-to-Globulin Ratio (AGR)

Malign Mezotelyomada Potansiyel Yeni Prognostik Faktörler: Sistemik İnflamatuar İndeksler (SII) ve Albümin-Globulin Oranı (AGO)

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ABSTRACT

Introduction: Malignant mesothelioma (MM) is rare with poor prognosis and often diagnosed at advanced stage. Systemic inflammatory indices (SII) may have prognostic value in cancer. Albumin is a negative acute phase reactant. We evaluated the prognostic significance of SII and albumin to globulin ratio (AGR) in MM followed-up at a single institute.

Methods: Fifty-six MM patients who met the inclusion criteria at our oncology cenrter were included in the study. Patients aged over 18 years with pathologically confirmed malignant pleural and peritoneal mesothelioma and no secondary malignancy followed up at our center were included in the study. Laboratory parameters for estimation of SII and AGR at diagnosis were obtained from database. Those with active infection, which might affect these parameters, those with a medical history of steroid use were excluded from the study.

Results: Median follow-up was 13.5 months. Most of the patients were female (58.9%). Median overall survival (OS) was 13 months. Median OS was 16 months in the pleural mesothelioma group and 9 months in the peritoneal mesothelioma group (p=0.982). Median OS was longer with lower platelet level, lower neutrophil to lymphocyte ratio (NLR) level and lower platelet to lymphocyte ratio (PLR) level (p1=0.001, p2=0.001 p3<0.001; respectively). On the other hand, median OS was longer with higher lymphocyte count, higher albumin level and higher AGR level (p1=0.032, p2=0.03, p3=0.003). Lymphocyte, Platelet count and AGR were determined as independent prognostic factors for OS according to multivariate cox regression analysis (p1=0.047, HR: 0.852; p2=0.011, HR: 2.502; p3=0.032, HR: 0.495, respectyively).

Discussion and Conclusion: It has been demonstrated that AGR, platelet and lymphocyte counts are independent prognostic factors for OS in MM.

Keywords: Malignant Mesothelioma, albumin, albumin-to-globulin ratio, systemic inflammatory indices (SII), NLR, PLR

ÖZET

Giriş ve Amaç: Malign mezotelyoma (MM) sıklıkla ileri evrede tanı alan kötü prognozlu nadir bir hastalıktır. Sistemik inflamatuar indeksler (SII) kanserde prognostik değerlere sahip olabilir. Albumin, negatif bir akut faz reaktanıdır. Sunulan calışmada MM takibinde SII ve albuminin globulin oranının (AGO) prognostik önemini değerlendirdik.

Yöntem ve Gereçler: Merkezimizde dahil edilme kriterlerini karşılayan 56 MM hastası calışmaya dahil edildi. 18 yaş üstü, patolojik olarak doğrulanmış malign plevral ve peritoneal mezotelyoma olan

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sekonder malignitesi olmayan hastalar calışmaya dahil edildi. Tanı anında SII ve AGR laboratuvar parametreleri veri tabanından retrospektif olarak kaydedildi. Bu parametreleri etkileyebilecek aktif enfeksiyonu olanlar, steroid kullaım öyküsü olanlar çalışma dışı bırakıldı.
Bulgular: Medyan takip süresi 13,5 aydı. Hastaların çoğunluğu (%58.9) kadındı. Medyan genel sağkalım (OS) 13 aydı. Median OS, plevral mezotelyoma grubunda 16 ay ve peritoneal mezotelyoma grubunda 9 aydı (p=0.982). Median OS, düşük trombosit seviyeleri, düşük nötrofil lenfosit oranı (NLR) seviyeleri ve düşük trombosit/lenfosit oranı (PLR) seviyelerinde daha uzundu (sırasıyla p1=0.001, p2=0.001 p3<0.001). Öte yandan, medyan OS yüksek lenfosit sayısı, daha yüksek albumin düzeyi ve daha yüksek AGR düzeyleriyle daha uzundu (p1=0.032, p2=0.03, p3=0.003). Lenfosit, trombosit sayısı ve AGR, multivariate cox regresyon analizine gore OS için bağımsız prognostik faktörler olarak belirlendi (p1=0.047, HR: 0.852; p2=0.011, HR: 2.502; p3=0.032, HR: 0.495, sırasıyla).
Tartışma ve Sonuç: Çalışmada AGO, trombosit ve lenfosit sayılarının MM'de OS için bağımsız prognostik faktörler olduğu gösterilmiştir.

Anahtar Kelimeler: Malign Mezotelyoma, albumin, albumin globulin oranı, sistemik inflamatuar indeksler, NLR, PLR

Introduction

Malignant mesothelioma (MM) is a rare neoplasm of serous membranes such as pleura, peritoneum, pericardium, and tunica albuginea [1]. It has poor prognosis with a median overall survival (OS) of around one year (range: 6-12 months) [2]. The incidence of pleural MM is approximately 10 to 30 fold higher than peritoneal MM [3]. The incidence is increasing worldwide, mainly due to occupational asbestos exposure [4]. There is a strong positive correlation between asbestos exposure and MM development at any localization. Respiratory exposure to asbestos has been reported as the main cause of pleural MM that accounts for approximately 70% of pleural MM cases who were documented for asbestos exposure [5].

Major histological subtypes are epithelioid, sarcomatoid, and biphasic (mixed) MM. Sarcomatoid MM has worse prognosis than epitheloid subtype [6]. 60% of MM patients present with stage III or IV disase at diagnosis [7]. In the literature, some factors including blood hemoglobin level and white blood cell count, Eastern Cooperative Oncology Group (ECOG) performance score and baseline symptoms have been reported to have prognostic significance [8,9]. However, their role as prognostic factors in MM are not so clear since most of clinical data are based on retrospective series in the literature because of its rarity and geographical distribution. Turkey, especially some regions such as Tuzköy and its nearby localizations tend to have relatively higher risk for MM because of erionit and others similar to asbestos structure in that region that may have role in development of MM [10]. Therefore, we should focus on MM in Turkey in terms of prognostic and predictive factors in MM.

In recent years, numerous studies, which have been conducted with inflammation-based markers, have obtained promising outcomes for revealing the prognosis in various cancers [11]. It has been demonstrated that systemic inflammation is associated with poor survival in many cancer types [12]. Inflammatory cells in the tumor microenvironment were shown to have significant effects on tumor development, and systemic inflammation blood markers may provide considerable information in predicting the prognosis [9]. Albumin and globulin are proteins that are the main component of serum. Albumin is a negative acute phase reactant which also reflects the nutritional status and systemic inflammatory response in cancer patients [13]. Globulin, the other main protein component of serum, has crucial roles in immunity and

inflammation [14]. Lower serum albumin level accompanied with higher globulin level may reflect inflammatory response in tumors. Recently, albumin to globulin ratio (AGR) has been reported to have prognostic value in various cancers [15]. However, its role has not been well studied in relatively rare tumors including MM. Therefore, we considered that AGR may have prognostic role in MM, besides other systemic inflammatory indeces such as neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR).

Hence, this study was planned to determine the prognostic factors which impact OS, by assessing the retrospective data of patients with MM at our center, who had been followed up in a single center, in the light of the literature.

Material Methods

Upon retrospectively reviewing the data of 102 MM patients who were followed up in our cancer center between 2011 and 2020, 56 patients met the inclusion criteria for our study were included. Patients aged over 18 years with pathologically confirmed malignant pleural and peritoneal mesothelioma without any secondary malignancy were enrolled. Laboratory parameters for SII and AGR at diagnosis were obtained from the patients' database. Those with active infection or a medical history of steroid use, which might affect these parameters, were excluded from the study. The demographic data of the patients and their clinical characteristics were noted down from the patient files. During the study follow-up, disease-free survival (DFS) and progression-free survival (PFS) were calculated based on the recurrence in patients with early-stage and progression in patients with advanced stages. Moreover, OS was calculated by using the central record, according to the dates of the deaths (death notification form). NLR, PLR and AGR were calculated with the formula: Neutrophil count (/μL) / Lymphocyte count (/μL); Platelet count (10^9/L) / Lymphocyte count (/μL) and Albumin value (g/dl) / Globulin value (g/dl). The study was approved by the local ethics committe, ethical approvel number 2021-04/1125; approvel date:21/04/2021.

Statistical Analysis

Statistical analyzes were performed via the software of SPSS 25.0 (SPSS, Chicago, IL, USA). Mann-Whitney U test was used for comparison of nonparametric data, and Student T-test was used for comparison of parametric data. Chi-Square or Fisher's Exact test was used for comparison of categorical data. Optimum cut-off values that can be used to determine the prognostic significance of NLR, PLR, AGR, lymphocyte count and platelet count were determined by receiver operating characteristic (ROC) analysis. Kaplan–Meier method was used for survival analysis, and the Log-Rank test was used for the comparisons between groups. Prognostic factors affecting overall survival were determined by conducting multivariate analysis with the Cox proportional hazards model. Variables with a p value under 0.20 as a result of univariate analysis were evaluated in the cox-regression model. The results were considered statistically significant at $p<0.05$.

Results

Thirty-three (58.9%) of 56 patients in the study were female. Median age of the patients was 65 years (18-77). While 37 (66.1%) patients had pleural mesothelioma, 19 (33.9%) patients were diagnosed with peritoneal mesothelioma. The demographic and clinical characteristics of the patients are summarized in Table-1. At the time of diagnosis, 18 patients (32.1%) were operable, 34 patients (60.7%) were unresectable, and 4 patients (7.1%) were medically inoperable. Pathologically, 40 patients (71.4%) had epithelioid MM while 9 patients (16.1%) had

Table 1: Summary of Patient Characteristics

Characteristics		
Gender		
	Female	33 (58.9%)
	Male	23 (41.1%)
Median Age		65 (18-77)
Tumor Location		
	Plevra	37 (66.1%)
	Periton	19 (33.9%)
Asbest Exposure		
	No	16 (28.6%)
	Yes	16 (28.6%)
	Unknown	24 (42.9%)
Smoker		
	No	39 (69.6%)
	Yes	17 (30.4%)
ECOG PS		
	<2	33 (58.9%)
	≥2	23 (41.1%)
Symptoms at diagnosis		
	Localized pain	39 (69.6%)
	Dyspnea	32 (57.1%)
	Weight loss	11 (19.6)
	Fatigue	28 (50%)

ECOG PS: Eastern Cooperative Oncology Group Performance Status

biphasic MM and 7 patients (12.5%) had sarcomatoid MM subtypes. Grade 3-4 adverse effects related to chemotherapy occurred in 17 patients (30.4%). Five pleural MM patients (8.9%) received adjuvant radiotherapy. Pathological, surgical and medical treatment characteristics of the patients are summarized in Table-2.

Median follow-up period was 13.5 months. In the study, median OS was 13 months (95% CI =9.85-16.14). Median OS was 16 months (95% CI=10.04-21.95) in the pleural MM group while it was 9 months (95% CI = 6.38-11.61) in the peritoneal MM group and there was no significant difference for OS between these two groups (p=0.982). Median DFS of 18 patients who recurred after surgery was 12 months (95% CI=4.16-19.84). In 38 non-operated patients, median PFS was 7 months (95% CI=4.93-9.06) following first line treatment. Median PFS was 8 months (95% CI = 4.04-11.98) for pleural MM group, and it was 6 months (3.41-8.58) for peritoneal MM group, as well. (p=0.159) (Table-3).

Table 2: Baseline Characteristics of Surgery Pathology and Therapy

Characteristics	
Operation	
Operable	18 (32.1%)
Inoperable	34 (60.7%)
Medical Inoperable	4 (7.1%)
Type of Operation	
Pleurectomy/Decortication (Pleural)	8 (14.3%)
Debulking (Peritoneal)	10 (17.9%)
HIPEC	4 (7.1%)
Pathology	
Epithelioid	40 (71.4%)
Sarcomatous	9 (16.1%)
Biphasic	7 (12.5%)
IHC Staining	
Kalretinin	56 (100 %)
WT-1	43 (75.57%)
Cytokeratin 5/6	33 (58.9%)
Chemotherapy at Adjuvant in Operable Patients	
Platin + Pemetrexed	14 (77.8%)
Platin+Pemetrexed+Bevacizumab	1 (5.6%)
Not Received	3(16.7%)
Chemotherapy at First Line in Inoperable Patients	
Platin + Pemetrexed	23 (60.5%)
Platin+Pemetrexed+Bevacizumab	5 (13.2%)
Platin + Gemcitabine	1 (2.6%)
Not Received	3(7.9%)
Unknown	6(15.8%)
Chemotherapy at Relapse in Operable Patients	
Pemetrexed	2 (28.5%)
Vinorelbine	1 (14.2%)
Gemcitabine	3 (42.8%)
Not Received	1 (14.2%)
Chemotherapy at Progression in Inoperable Patients	
Gemcitabine	13 (46.4%)
Platin + Gemcitabine	1 (3.5%)
Pemetrexed	2 (7.14%)
Vinorelbine	1 (3.5%)
Not Received	11(39.2%)
Adjuvant Radiotherapy	5 (8.9%)

HIPEC: Hyperthermic Intraperitoneal Chemotherapy

Table-3: Survival Rates

	OS (month)
All patients (n:56)	13 (9.85-16.14)
Pleural	16 (10.04-21.95)
Peritoneal	9 (6.38-11.61)
	DFS (month)
Recurrence after surgery (n:18)	12 (4.16-19.84)
	First Line PFS (month)
Non operated patients (n:38)	7 (4.93-9.06)
pleural	8 (4.04-11.98)
peritoneal	6 (3.41-8.58)

The patients with better ECOG-PS at diagnosis had longer OS. Median OS was 22 months (95% CI=16.56-27-44) for the patients with an ECOG-PS <1 at the time of diagnosis whilst it was 7 months (95% CI=3.79-10.22) for the others with ECOG-PS >2 and the difference between these two groups was statistically significant (p=0.002). While median OS was 22 months (95% CI =7.53-36.46) in the operated patients, it was 11 months (95% CI=6.97-15.02) in the non-operated patients (p=0.014). Of the patients who had progression with first line treatment, 17 patients were followed-up with best supportive care (BSC) whereas 6 patients had second line chemotherapy. When these subgroups were compared for PFS, those who had second line chemotherapy had numerically longer PFS, however, the difference was not statistically different (6 months versus 3 months, p=0.141).

Optimum cut-off values for NLR, PLR, AGR, albumin, lymphocyte count, and platelet count which predicting OS were 3.0, 200, 1.07, 3.5, 1500, and 350, respectively. Median OS was 22 months (95% CI = 7.43-36.56) for the patients with lower NLR level while it was 9 months (95% CI = 4.00-13.99) for the patients with higher NLR level (p=0.001). Median OS was 26 months (95% CI = 11.66-40.33) for the patients with lower PLR level while it was 9 months (95% CI = 4.01-13.98) for the patients with higher PLR level (p<0.001). Median OS was 9 months (95% CI= 4.15-13.84) for the patients with lower AGR level while it was 22 months (95% CI =13.33-30.66) for the patients with higher AGR level (p=0.003). OS was significantly longer in the patients with higher albumin level (>3.5 g/dL). Median OS was 19 months (95% CI = 11.21-26.78) for the patients with an albumin value of >3.5g/dL, while it was 9 months (95% CI = 6.24-11.75) for the patients with an albumin value of ≤3.5 g/dL (p=0.03). OS was significantly longer in the patients with higher lymphocyte level (18 vs 10 months, respectively; p=0.032) while it was significantly shorter in the patients with higher platelet level (10 vs 28 months, respectively; p=0.001).

Multivariate cox regression analysis was performed with lymphocyte, AGR, albumin, NLR, PLR and Platelet. Lymphocyte, Platelet count and AGR were determined as independent prognostic factors for OS according to multivariate cox regression analysis [p=0.047, HR: 0.852 (95% CI= 0.674 –0.986) for lymphocyte count; p=0.011, HR: 2.502 (95% CI =1.233 – 5.076) for platelet count; p=0.032, HR: 0.495 (95% CI =0.260–0.942) for AGR, respectyively].

Discussion

In this study, in which 56 patients with malignant pleural and peritoneal MM were investigated retrospectively in a single cancer center, it was demonstrated that lymphocyte, AGR values and platelet count at the time of diagnosis are independent prognostic factors for OS. Median age of was 65 years, and when the literature was reviewed, it was found to be higher compared to other studies that have been conducted in Turkey [1,16].

Median OS was 13 months for all population. It increased to 16 months in the pleural MM subgroup and whereas it decreased to 9 months in the peritoneal MM group. It is well-

known that OS varies depending on the clinical characteristics of the patients with MM, such as stage at diagnosis, operability and pathological subgroups. In the series in which Dogan et al. examined patients with pleural and peritoneal MM, median OS was 22 months, while in a large series of 910 patients in which only patients with pleural MM were evaluated, median OS was determined to be 10 months [1,17]. Besides, in a study conducted on patients with peritoneal MM, median OS was determined as 11 months [18].

In the present study, the patients who were operated had a significant survival advantage when compared to those who were not operated (22 months vs. 11 months). It is well-documented that median OS in patients with pleural MM who underwent extrapleural pneumonectomy is 18 months and that a substantial number of patients achieve long-term survival [19]. In a study in which 27 patients with peritoneal MM were given intraperitoneal chemotherapy in addition to cytoreductive surgery, the 3-year survival was determined to be 67% [20]. Unlike this study, in presented study 10 out of 19 peritoneal MM patients were operated and only 4 received intraperitoneal chemotherapy.

It is well-established that the ECOG performance score is a prognostic factor in various cancers [21]. In a Taiwan study, which was conducted on patients with pleural MM, it was revealed that patients with an ECOG performance score of ≥2 had a poor prognosis [22]. Consistent with the literature, patients with low ECOG performance scores had a shorter OS in the presented study (22 months vs. 7 months). These differences in survival outcomes might be related to the heterogeneity of the studies, including the fact that some of them are retrospective, the number of patients, ECOG performance scores, pathological subtypes, operation status, whether intraperitoneal chemotherapy is given in peritoneal mesothelioma, and the difference in the chemotherapy protocols.

In the study, when the albumin levels > 3.5 and AGR >1.07 by using the ROC curve, the median OS was statistically significant at high albumin and AGR levels. Also, AGR was independent prognostic factors for OS. It is well-known that serum albumin level, which is simple, inexpensive, and widely available, is a negative acute phase reactant and decreases as inflammation increases [23]. Furthermore, as malnutrition is very common in cancer patients, serum albumin level is often used to assess malnutrition status [24]. It has been demonstrated in a study, which was conducted on various cancers, that serum albumin level is an independent predictive marker indicating malnutrition [25,26]. Total serum protein and albumin show the absorption, synthesis, and decomposition of body proteins. Moreover, albumin has anti-tumor activity and can reflect immune system functions into practice [27]. Globulin, which is the other major protein component of serum may rise in serum as a result of the accumulation of acute-phase proteins which are involved in inflammation [28]. Studies have found out that increased cytokines in cancers are associated with a rise in immunoglobulin. This situation corroborates the thesis that an elevated level of globulin may be associated with apoptosis inhibition and cancer progression [29]. Hence, the AGR derived from albumin and globulin could be used as a factor indicating cancer progression [28]. Considering these data, it was found out that increasing serum AGR before treatment in patients with malignant mesothelioma were associated with better survival, additionally AGR was also an independent prognostic factor for OS. Consistent with the presented study, pre-treatment low AGR was reported to be significantly associated with poorer OS, increased 5-year mortality rates besides higher relaps and progression rates in a meta-analysis of 15356 patients diagnosed with

various cancer types such as gastric cancer, colorectal cancer, breast cancer, larynx carcinoma, and hepatocellular cancer [15]. Moreover, the studies including many solid tumor types at different stages demonstrated that basal AGR at diagnosis was associated with a better OS, DFS and PFS [30,31,32].

It is well-documented that hypoalbuminemia is a poor prognostic factor in many cancers [33,34]. In the presented study, a significant OS difference was determined between the patients with a serum albumin level of >3.5g/dL and those with a level of ≤3.5 g/dL (22 months vs. 9 months). In paralel to this data, pre-treatment serum albumin level was defined as an independent prognostic factor for OS in pleural MM [25]. Consistent with the presented study, studies performed in the patients with peritoneal MM also revealed poorer overall survival as the serum albumin value decreased [18, 24].

In the study, when the cut-off values for NLR, PLR, platelet and lymphocyte were taken using the ROC curve, the median OS was statistically significantly lower at high NLR, PLR and platelet levels, while the median OS was statistically significant at high lymphocyte levels. Besides platelet and lymphocyte markers were independent predictive factors for prognosis. The efficacy of these blood inflammatory parameters has been reported in numerous studies conducted on patients with MM. In a meta-analysis of 1533 pleural MM patients, it was revealed that increased NLR was associated with poorer survival rates [9]. In another study, PLR was also determinned to have prognostic significance [35]. It is well-known that platelets have a crucial role in inflammation and have a prognostic significance [36]. In a meta-analysis, pre-treatment high platelet count was shown to be associated with a poorer OS [37]. Lymphocytes act as tumor suppressors by inducing cytotoxic cell death and inhibiting tumor cell proliferation and migration [38]. Tumor-infiltrating lymphocytes can activate an effective antitumor cellular immune response [39]. Thus, as demonstrated in the presented study, increased lymphocyte counts may be associated with better survival outcomes.

This study has some limitations. It was retrospective, and a prospective multicenter study would be much better in terms of evaluating the prognostic factors of malignant mesothelioma. In this study, there is a risk of bias in some results due to the lower number of patients and missing data.

Conclusions

In this study, it has been demonstrated that AGR, platelet and lymphocyte counts are independent prognostic factors in MM. Higher albumin levels and AGR are associated with better survival. Large prospective clinical trials will provide better information and could reduce the possibility of bias.

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Case Report

MRI Findings of Infundibular Craniopharyngioma: Two Case Reports

İnfundibular Kraniofaringiomanın MR Görüntüleme Bulguları: İki Olgu Sunumu

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ABSTRACT

Primary infundibular craniopharyngioma is a relatively unusual disease due to its location, which usually results in late diagnosis. Two female patients are referred to the radiology clinics because of bitemporal hemianopsia detected in visual assessment at different times.

Infundibular lesions were detected with MRI both patients and craniopharyngioma was considered as the primary diagnosis. Both masses are operated and the diagnosis of craniopharyngioma is proven pathologically.

Keywords: MRI, infundibular Craniopharyngioma, Pituitary infindubulum

ÖZET

Primer infundibular kraniofarenjiyoma, konumu nedeniyle nispeten nadir görülen ve genellikle geç tanı alan bir hastalıktır. Farklı zamanlarda norolojik muayenelerinde bitemporal hemianopsi tespit edilen iki hasta MRG incelemesi için radyoloji kliniğine refere edildi. Her iki hastada da MRG ile infindubüler yerleşimli lezyonlar tespit edildi ve kraniofaranjiyom birincil tanı olarak düşünüldü. Her iki kitle de ameliyat edildi ve kraniofaranjiyom tanısı patolojik olarak doğrulandı.

Anahtar Kelimeler: MRG, İnfundibular Kraniofaringioma, Hipofizer İnfundibulum

Introduction

Craniopharyngiomas account for 1–4% of all intracranial tumours and 20% of the tumours of the sellar and chiasmatic region [1]. The tumor has two age peaks, the one occuring in children and the other one in adults between the 4th and 6th decades despite it's more common in childhood than adulthood period. Craniopharyngiomas arise from remnants of embryonic canal extending from the oropharynx to the median eminence and infundibulum. According to this theory, any place along this canal may serve as a site of tumor origin [2]. Although the epicenter of the lesions is usually suprasellar (90%), sellar or infrasellar region; anterior, middle and posterior cranial fossas, retroclival region, sphenoid bone, nasopharynx, cerebello-pontine angle and even pineal gland are other rare sites of the tumor development [3]. Infundibulum along the embryonic canal is another potential site for tumor origin. However, primary infundibular cranio-pharyngioma is a rare disease and uncommonly early diagnosed until it grows towards the suprasellar or parasellar regions. It usually presents with neurological complications like headache, visual disturbance and symptoms related with hypothalamic or hypophyseal gland dysfunction [4].

Here we report unusual infundibular cranio-

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pharyngioma cases which is pathologically confirmed and primarily arising from the infundibulum. We want to discuss imaging findings and differential diagnosis of infundibular craniopharyngioma.

Case Report

Case 1: A 32-year-old female patient has been admitted to the neurology outpatient clinic with complaints of persistent vision problems and headache for one month. Bitemporal hemianopsia is detected in the visual examination.

CT showed a hypodens mass with punctate peripheral calcifications in the suprasellar area. MRI revealed that the mass was originating from the pituitary infindubulum and containing cystic and solid areas, approximately 20x20 mm in size. The lesion compresses the optic chiasm posteriorly and is closely adjacent to vascular structures. The pituitary gland had a normal appearance. Based on these findings, radiological diagnosis was considered to be infindubular craniopharangioma (Figure 1a-c). Infundibular craniopharyngioma was confirmed histo-pathologically. On the 2nd year follow-up MRI, there was a mass lesion measuring 15 x 10 mm consistent with residual or recurrent tumor(Figure 1d).

Case 2: A 61-year-old woman suffering from visual disturbance for 2 months admitted to the hospital. Visual examination of the patient revealed bitemporal hemianopsia. An infundibular mass was demonstrated as intermediate signal intensity on T1-weighted MR images and high signal intensity similar to CSF corresponding to cystic changes on T2-weighted images. It showed peripheral and nodular enhancement pattern on postcontrast T1-weighted images. No restriction of diffusion is noted on diffusion weighted images. The tumor was extending from hypophyseal infundibulum to hypothalamus and compressing optic chiasm. (Figure 2 a-c) The initial radiological diagnosis of the lesion was craniopharyngioma, and then the lesion was decided to be operated on. Histopathological results confirmed infundibular craniopharyngioma. Follow-up MRI images two months after the operation showed no recurrence or residual tumor.

Discussion

Craniopharyngiomas are histologically benign tumors orginating from squamous epithelial cells of Rahtke's cleft. They usually involve sellar-suprasellar region and invade or extend to clinically important structures as optic chiasm and hypothalamus. It is more common in childhood than adulthood period. However our two patients were adult. Although craniopharyngiomas usually arise from Rathke's cleft, they can also arise from embryonic cells located anywhere along the craniopharyngeal canal. [5].

Infundibular involvement may be seen in many conditions as well as in craniopharyngiomas. It is important to make a differential diagnosis of infundibular diseases in order to treat appropriately. Infundibular lesions are generally classified into three categories as neoplastic, inflammatory-infectious and congenital-developmental. Neoplastic lesions include astrocytoma, ependymoma, germinoma, pleomorphic xanthoastrocytoma, lymphoma, prolactinoma, metastasis and craniopharyngioma. The first diagnosis to be considered in the absence of primary tumor in isolated stalk masses is craniopharyngioma.The size of infundibulum usually should be lesser than basilar artery at clivus level. More than this size might be a remarkable point for infundibular lesions. In many cases, hypothalamus is involved together with the infundibulum. The lesions involving both pituitary stalk and hypothalamus may result in diabetes insipidus.

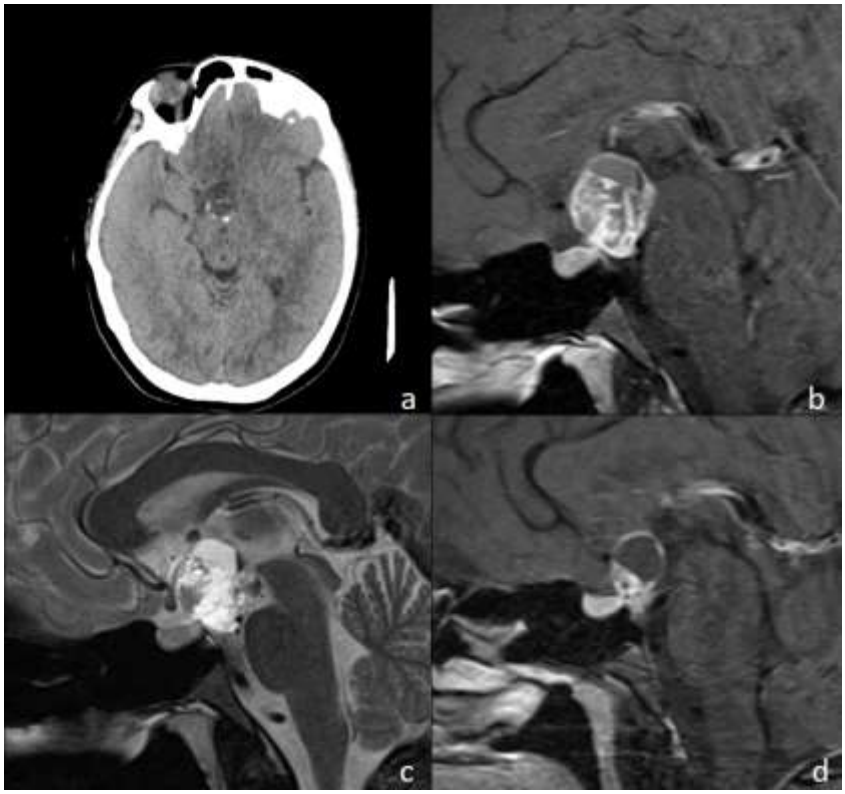

Figure 1(a-d):Non-contrast CT shows a mass with peripheral punctate calcifications in the suprasellar area. (a).Solid lesion of stalk with cystic-necrotic component is shown on contrast enhanced T1-weighted sagittal image(b) and T2-weighted sagittal image(c).The mass copresses the optic chiasm from the posterior and causes visual symptoms(c).The remaining mass is demonstrated in the postop contrast-enhanced MR image taken two years after surgery(d).

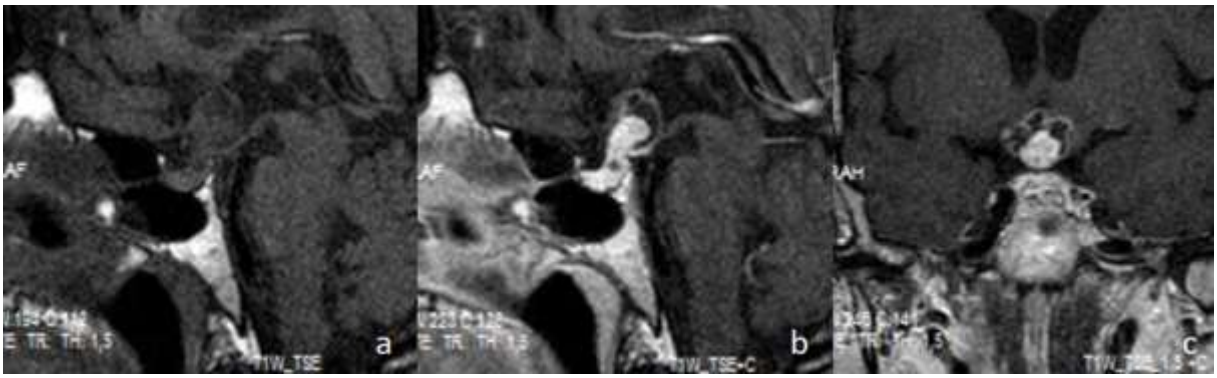

Figure 2(a-c). Sagittal non-contrast T1-weighted MRI shows heterogeneous suprasellar mass lesion (a). Contrast-enhanced solid lesion of stalk with superior cystic-necrotic component demonstrates on contrast enhanced T1-weighted sagittal (b) and coronal (c) images.

Pituitary adenomas may cause other endocrinologic problems due to excessive secretion of hormones. Germinomas which usually present as a tumor of pineal gland or hypothalamus, can also be seen in primarily infundibulum [6]. Metastasis to infundibulum are usually from breast and lung cancers. In the presence of primary tumor, metastasis can be considered primarily, but neither of our patients had primary tumors. Leukaemia, lymphoma and atypical/malignant meningiomas which are aggressive tumors of

hypothalamic-suprasellar region are other rare tumors can involve infundibulum [1]. Ratke's cleft cysts should be taken into accont if there is a cystic tumor of infundibulum. All of these tumors are in the differential diagnosis of infundibular craniopharyngiomas. However, there are some granulomatous diseases of the infundibulum that may resemble neoplasms. Sarcoidosis, tubercu-losis, langerhans cell histiocytosis are such kind of granulomatous diseases that involve infundibular stalk. Therefore, MRI is an important imaging method in the differential diagnosis of infundibular lesions.

In our cases, infundibular involvement is due to a true neoplastic pathology called infundibular craniopharyngioma. Visual impairment is one of the major and earliest presenting symptoms of patients harboring craniopharyngiomas and also a potential complication of the surgical treatment [7]. The first symptom in our patients was visual problems.Also it is usually diagnosed late unless complains of headache, polyuria and polydipsia or complains due to compression of optic chiasm consist. After diagnosis of craniopharyngioma, the next step is to choose appropriate treatment modality. There is a therapeutic dilemma for craniopharyngioma, so the approach is important when deciding on conservative or aggressive treatment. Conservative treatment may be an alternative management for early diagnosed small tumors especially for the tumors which are not extending to optic chiasm. However, there is no consensus on treatment options of larger lesions extending to optic chiasm. Complete surgical resection is usually indicated because it is believed that craniopharyngiomas are curable tumors. It is important to conserve infundibulum but it is sometimes sacrificed for total resection. Another alternative treatment modality for some authors is limited surgery followed by radiotherapy [8,9]. Surgical treatment was preferred in both of our patients. While total resection was achieved in our second case, our first case is being followed due to a residual mass.

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Original Article

Hereditary Breast-Ovarian Cancer and BRCA1/BRCA2 Variants: A Single Center Experience

Herediter Meme-Over Kanseri ve BRCA1/BRCA2 Varyantları: Tek Merkez Deneyimi

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ABSTRACT

Objective: In this study, it was aimed to determine the frequency of BRCA1 and BRCA2 variants in patients admitted to our clinic with hereditary breast-ovarian cancer and / or family history and to evaluate them in the light of the literature.

Materials and Methods: All patients in our study were selected according to the current NCCN guideline test criteria. The Ion Torrent ™ Oncomine ™ BRCA Research Assay was used to sequence the coding regions of the BRCA1 and BRCA2 genes in our patients. In addition, all patients with copy number changes were confirmed with SALSA® MLPA® Probemix P002 BRCA1 and Probemix P090 BRCA2 (MRC Holland).

Results: Variants (pathogenic, likely pathogenic, variants of uncertain clinical significance, and copy number variations) were detected in 39 of the 149 patients included in the study. Novel variants that were not previously described in the literature were detected in two patients, one of the BRCA1 and one of the BRCA2 gene, respectively.

Conclusion: In our study, the incidence of BRCA1 and BRCA2 variants was found to be 26.1%. This rate was higher than previous studies conducted in Turkey. Further studies are needed to identify common variants in the Turkish population and to evaluate the pathogenity of variants of uncertain clinical significance.

Keywords: Hereditary cancer, breast cancer, ovarian cancer, BRCA1, BRCA2

ÖZET

Amaç: Bu çalışmada kliniğimize herediter meme-over kanseri ve/veya aile öyküsü nedeniyle başvuran hastalardaki BRCA1 ve BRCA2 varyantlarının sıklığının tespiti ve literatür eşliğinde değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Çalışmamızdaki tüm hastalar güncel NCCN rehberi test kriterleri doğrultusunda seçilerek dahil edilmiştir. Hastalarımızda BRCA1 ve BRCA2 genlerinin kodlayıcı bölgelerini dizilemek için Ion Torrent™ Oncomine™ BRCA Research Assay kullanılmıştır. Ayrıca kopya sayısı değişiklikleri tespit edilen tüm hastalar SALSA® MLPA® Probemix P002 BRCA1 ve Probemix P090 BRCA2 (MRC Holland) ile konfirme edildi.

Bulgular: Çalışmaya dahil edilen toplam 149 hastanın 39'unda varyantlar (patojenik, muhtemel patojenik, klinik önemi belirsiz varyantlar ve kopya sayısı değişiklikleri) tespit edilmiştir. İki hastamızda (Biri BRCA1 geninde, biri BRCA2 geninde) daha önce literatürde tanımlanmamış yeni varyantlar tespit edilmiştir.

Sonuç: Çalışmamızda BRCA1 ve BRCA2 varyantlarının görülme sıklığı %26,1 olarak belirlendi. Bu oran Türkiye'de yapılan önceki çalışmalara göre daha yüksek bulundu. Türk toplumundaki sık varyantların ve özellikle klinik önemi belirsiz varyantların patojenitesinin daha net değerlendirilebilmesi için daha fazla çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Herediter kanser, meme kanseri, over kanseri, BRCA1, BRCA2

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Introduction

According to the WHO 2020 records, more than 2,250,000 individuals have been diagnosed with breast cancer and the breast cancer has become the most common type of cancer in the world. More than 300,000 women were also diagnosed with ovarian cancer[1]. Many molecular pathways, both genetically and epigenetically, play role in the etiopathogenesis of breast cancer and ovarian cancer. Both cancers show genetic heterogeneity in terms of clinical and biological features. Most cancer cases are considered to be sporadic appearing tumors because there is no clear family history, but cancer syndromes with a known genetic cause or hereditary predisposition to cancer have also been identified [2]. Individuals carrying an inherited genetic mutation and epigenetic abnormalities in tumor suppressor genes have an increased risk of developing cancer throughout their lifetime. Germline mutations in cancer susceptibility genes cause cancer if the normal allele is lost or inactivated. Breast and ovarian cancers (5-10%) can be inherited and occur with cancer-prone syndromes [3].

There are many genes that can increase the risk of developing breast and/or ovarian cancer. In the early 1990s, the BRCA1 and BRCA2 genes were identified as associated with breast and ovarian cancer [4, 5]. Hereditary breast and ovarian cancer (HBOC) caused by pathogenic variants in the BRCA1 and BRCA2 genes is the best known and most common form. It occurs in all ethnic populations. The prevalence of BRCA1/2 pathogenic variants in the population is estimated to be 1/400 to 1/500 [6]. International guidelines such as NCCN state that patients with suspected hereditary breast cancer and all women with epithelial ovarian cancer should seek genetic counseling and comprehensive genetic testing should be recommended. In centers with suitable conditions, patients should be directed to genetic counselors. Before genetic tests, a comprehensive risk assessment should be done to patients and their relatives.

HBOC is a well-known cancer syndrome in which BRCA pathogenic variants are responsible for up to 80% [7]. In addition, high risk gene variants (PALB2, TP53, PTEN) or intermediate risk gene variants (ATM, CHEK2) are also associated with HBOC syndrome [8]. According to a metaanalysis study, individuals with HBOC syndrome have a lifetime risk of developing ovarian cancer (40% for BRCA1 variants and 18% for BRCA2 variants by the age of 70) and/or breast cancer (57% for BRCA1 variants and 49% for BRCA2 variants by the age of 70) [9]. Genetic testing is now widely recommended in cancer diagnosis all around the world and may have the potential to influence treatment decisions. For example, current guidelines recommend the use of poly ADP-Ribose polymerase inhibitors (PARPi) in treatment protocols for patients with BRCA 1/2-related cancer [10]. Therefore, it is very important to determine cancer-related genetic etiology in patients. Identifying pathogenic variant carriers and individuals at risk may reduce morbidity and mortality from cancer. Identifying pathogenic variants in at-risk individuals, it may significantly influence disease course by giving individuals the opportunity to evaluate risk-reducing strategies, such as enhanced surveillance, variant-specific next-generation treatments or surgical interventions.

The National Comprehensive Cancer Network (NCCN) has published recommendations to assist clinicians in identifying individuals with hereditary cancer syndrome, and these recommendations are frequently updated. In the presence of any of the following criteria, there is an indication for genetic testing for hereditary breast, ovarian and pancreatic cancer (Table-1) [11].

After genetic counseling and a comprehensive risk assessment, there may be differences in

Table 1. Testing criteria for breast and/or ovarian cancer susceptibility genes

1.	Individuals with any blood relative with a known Pathogenic/Likely pathogenic variant in a cancer susceptibility gene
2.	Individuals meeting the criteria below but tested negative with previous limited testing (eg, single gene and/or absent deletion duplication analysis) interested in pursuing multigene testing
3.	Personal history of cancer • Breast cancer with at least one of the following ➢ Diagnosed at age ≤45 y; or ➢ Diagnosed at age 46-50 y with ▪ Unknown or limited family history; or ▪ A second breast cancer diagnosed at any age; or ▪ ≥1 close blood relative with breast, ovarian, pancreatic or prostate cancer at any age ➢ Diagnosed at age ≤60 y with triple-negative breast cancer ➢ Diagnosed at any age with; ▪ Ashkenazi Jewish ancestry; or ▪ ≥1 close blood relative with breast cancer at age ≤50 y ovarian, pancreatic, metastatic, intraductal/cribriform histology or high- or very-high-risk group prostate cancer at any age; or ▪ ≥3 total diagnoses of breast cancer in patient and/or close blood relatives ➢ Diagnosed at any age with male breast cancer • Epithelial ovarian cancer (including fallopian tube cancer or peritoneal cancer) at any age • Exocrine pancreatic cancer at any age • Prostate cancer at any age with ➢ metastatic, intraductal/cribriform histology or high- or very-high-risk group ➢ Any NCCN risk group with the following family history: ▪ Ashkenazi Jewish ancestry; or ▪ ≥1 close blood relative with breast cancer at age ≤50 y ovarian, pancreatic, metastatic or intraductal/cribriform prostate cancer at any age; or ▪ ≥2 close relative with either breast or prostate cancer (any grade) at any age • A mutation identified on tumor genomic testing that has clinical implications if also identified in the germline • Individuals who meet Li-Fraumeni syndrome testing criteria or Cowden syndrome/PTEN hamartoma tumor syndrome testing criteria • To aid in systemic therapy decision-making, such as for HER2-negative metastatic breast cancer
4.	Family history of cancer • An affected or unaffected individual with a first- or second-degree blood relative meeting any of the criteria listed above ➢ If the affected relative has pancreatic cancer or prostate cancer, only first-degree relatives should be offered testing unless indicated for other relatives based on additional family history • An affected or unaffected individual who otherwise does not meet the criteria above but has a probability ≥5% of a *BRCA1/2* pathogenic variant based on prior probability models.

choosing the appropriate test for individuals. Preferably, the analysis of BRCA1 and BRCA2 genes, which are known to be associated with hereditary breast and ovarian cancer, is generally a suitable option. However, with the advancing new technologies in recent years, multiple gene panels including BRCA1 and BRCA2 genes can be preferred at the first stage [12].

Material-Method

A total of 149 patients who applied to Başakşehir Çam and Sakura City Hospital, Medical Genetics Department between May 2020 and April 2021 were included in our study. Our study was conducted in accordance with the Declaration of Helsinki and was approved by ethics committee of Basaksehir Çam and Sakura City Hospital (KAEK/ 2021.04.57).

All included patients were selected according to the current NCCN genetic testing criteria in relation to hereditary breast-ovarian cancer syndrome. We examined the variants in the BRCA1 and BRCA2 genes via the next generation sequencing. In addition, we performed CNV analyzes of these genes and also confirmed with MLPA in some of our patients.

Genomic DNA was isolated from peripheral blood of patients after the completion of a consent form. DNA isolations were made from sterile 2 ml EDTA peripheral blood samples using PureLink ™ Genomic DNA Mini Kit. After DNA isolation, the densities of the DNA samples to be included in the study were measured with the Qubit dsDNA HS Assay Kit with the fluorometric method (Qubit® 4.0 Fluorometer), and the final DNA concentrations were ensured to be in the desired range (5-10 ng/µl) for next generation sequencing. Library preparation, BRCA1 and BRCA2 genes were amplified using Oncomine BRCA panel pools using Ion AmpliSeq Library Kit Plus (Life Technologies) in accordance with company protocols. Library products were created in 200 bp fragments. Library products were barcoded with the IonXpress Barcode Adapters Kit (Life Technologies). In order to remove other materials and enzymes from the barcoded samples, the unproduced library products were enzymatically purified by FuPa. At this stage, the application of emulsion PCR and enrichment processes to the normalized library products and the purification and sequencing of the enriched PCRs, the clonal reproduction of the library products containing the target regions by creating oil-water emulsions and loading them on the reading chips were performed on the Ion Chef ™ device. Ion AmpliSeq ™ Library Kit Plus was used for sequencing. In accordance with company protocols; for reading libraries on the Ion GeneStudio S5 Plus sequencer, the enriched products were loaded into the chip (Ion 520 ™ Chip) on the Ion Chef ™ instrument. After sequencing, the BAM files belonging to the data transferred to the Torrent server software were transferred to the Ion Reporter software for analysis. Each patient was analyzed with the programs Ion Reporter™ 5.16.0.3 and The Integrative Genomics Viewer (IGV) with this flowchart. The variants were classified according to the open access databases and ACMG guidelines, and their pathogenicity was determined. Sanger validation was performed for: homo-polymer regions, low quality variants, insertions and/or deletions, splice site alterations and novel variants. Since the reading depths of the samples are at least 300x, CNV (copy number variation) analysis for each sample was also analyzed with Ion Reporter ™ 5.16.0.3 program.

All CNVs detected via the analysis of next generation sequencing data were confirmed by MLPA. SALSA® MLPA® Probemix P002 BRCA1 (MRC Holland) and SALSA® MLPA® Probemix P090 BRCA2 (MRC Holland) were used in combination with a

SALSA MLPA reagent kit according to manufacturer's guidelines. CNV analysis was performed using Coffalyser.Net data analysis software.

Results

A total of 149 patients with breast and / or ovarian cancer or family history selected in line with the NCCN guidelines were included in our study. Variants (Pathogenic, likely pathogenic, VUS and CNVs) in BRCA1 and BRCA2 genes were detected in (26,1%) 39 of 149 patients. While a total of 14 different BRCA1 variants (single nucleotide variations and small indels) were detected in 17 patients, a total of 7 different BRCA2 variants were detected in 8 patients. When these 14 different BRCA1 variants were classified according to the ACMG guideline, 10 of these were considered pathogenic and four of these were considered variant of uncertain significance (VUS). When BRCA2 variants were evaluated according to the ACMG guideline, five of these were considered pathogenic and two of these were considered VUS. To the best of our knowledge, two novel variants were detected which have not been reported in the literature and public databases previously, one in the BRCA1 gene and one in the BRCA2 gene.

When the patients are evaluated according to the application reason, BRCA1 variants were found in 7 of 15 patients and BRCA2 variants were found in 8 of 15 patients tested for breast cancer. 9 patients were tested because of the ovarian cancer. BRCA1 variants were found in 7 of these patients and BRCA2 variants were found in two of these patients. The remaining five patients were evaluated because of their family history of cancer. BRCA1 variants were detected in four and BRCA2 variant in one of these patients.

In addition, as a result of CNV analysis, various deletions and duplications containing one or more exons in the BRCA1 and BRCA2 genes were detected in 11 patients. CNVs were detected in the BRCA1 gene in 7 individuals from five families. Also, CNV was detected in the BRCA2 gene in four individuals from the same family. All of these CNVs were confirmed by MLPA analysis. Detailed information about the patients detected variants are summarized in Table-2 and 3.

Discussion

In the literature, there are many studies conducted in many ethnic groups related to hereditary breast and ovarian cancer and BRCA1/ BRCA2 genes. The rate of detecting genetic variants in these studies varies according to the characteristics of the patients analyzed. For example, in a study of 517 patients in Jordan, pathogenic or likely pathogenic BRCA1 or BRCA2 variants were detected in 72 (13.9%) patients in the whole group in the BRCA1 (n=24, 4.6%) and BRCA2 (n = 48, 9.3%) genes, while VUS was reported in 53 (10.3%) patients [13].

Pathogenic BRCA1/ 2 variants were detected in 13 of 65 patients in a single-center study conducted in Japan in which individuals with triple negative breast cancer were examined. The reason for the small number of patients in the study was emphasized as that the BRCA1/ 2 genetic tests are not under the guarantee of the national health system [14].

There are also several studies conducted in different centers in Turkey associated with the BRCA1 and BRCA2 genes and HBOC recently. In the study of Solmaz et al. published in 2020, variants were detected in 85 of 910 (9.34%) patients selected according to the genetic test criteria in line with the NCCN guidelines. They have determined 31 different variants of the BRCA1 gene in 41 patients and 37 different variants of BRCA2 genes in 44 patients [15]. In another study in published in 2020 conducted in the Thrace region of Turkey, 39 different variants were identified in (17.8%) 88 out of a total of 493

Table 2. *BRCA1* and *BRCA2* sequence variants and pathogenicity classifications of our patients

Patient No	Age	Family History	Reason for application	Gene	Transcript	Location	cDNA change	Protein change	Varyant type	dbSNP	ACMG Classification
1	63	(-)	Ovarian cancer	BRCA1	NM_007300.4	Exon 4	c.181T>G	(p.Cys61Gly)	SNV	rs28897672	Pathogenic (PM1,PM2,PM5,PP3,PP5,BP1)
2	39	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 10	c.1259A>G	p.Asp420Gly	SNV	rs730881442	VUS (PM2)
3	39	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1286T>C	p.Ile429Thr	SNV	rs775869160	VUS (PM2,PM3,BP1)
4	38	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1286T>C	p.Ile429Thr	SNV	rs775869160	VUS (PM2,PM3,BP1)
5	37	(+)	Ovarian cancer history in the family	BRCA1	NM_007300.4	Exon 10	c.1504_1508delTTAAA	p.Leu502AlafsTer2	Deletion	rs80357888	Pathogenic (PVS1, PM2, PP3, PP5).
6	48	(-)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.2131_2132delAA	p.Lys711ValfsTer6	Deletion	rs398122653	Pathogenic (PVS1,PM2,PP5)
7	57	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 10	c.2666 C>T	p.Ser889Phe	SNV	rs769712441	VUS (PM2,BP1)
8	39	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.3333delA	p.Glu1112AsnfsTer5	Deletion	rs80357966	Pathogenic (PVS1,PM2,PP5)
9	51	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.3477_3480delAAAG	p.Ile1159MetfsTer50	Deletion	rs80357781	Pathogenic (PVS1,PM2,PP3,PP5)
10	60	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 10	c.4036delG	p.Glu1346LysfsTer20	Deletion	rs886040189	Pathogenic (PVS1,PM2,PP3,PP5)
11	24	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 12	c.4246G>C	p.Ala1416Pro	SNV	**Novel**	VUS(PM2,PP3,BP1)
12	72	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 18	c.5159G>A	p.Arg1720Gln	SNV	rs41293459	Pathogenic (PM1,PM2,PM5,PP3,PP5,BP1)
13	43	(+)	Breast Cancer	BRCA1	NM_007300.4	Intron 19	c.5256+1G>A	----------	SNV	rs80358004	Pathogenic (PVS1,PM2,PP3,PP5)
14	47	(+)	Breast Cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
15	42	(+)	Ovarian cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
16	45	(-)	Breast Cancer	BRCA1	NM_007300.4	Exon 20	c.5329dupC	p.Gln1777ProfsTer74	Insertion	rs80357906	Pathogenic (PVS1,PS3,PM2,PP3,PP5)
17	45	(+)	Multiple cancer history in the family	BRCA1	NM_007300.4	Exon 23	c.5507G>A	p.Trp1836Ter	SNV	rs80356962	Pathogenic (PVS1, PM2, PP2, PP3, PP5)
Patient No	**Age**	**Family History**	**Reason for application**	**Gene**	**Transcript**	**Location**	**cDNA change**	**Protein change**	**Varyant type**	**dbSNP**	**ACMG Classification**
1	37	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 5	c.469A>T	p.Lys157Ter	SNV	rs1593886887	Pathogenic (PVS1,PM2,PP3,PP5)
2	81	(-)	Breast Cancer + Pancreatic cancer	BRCA2	NM_000059.4	Exon 11	c.3318C>G	p.Ser1106Arg	SNV	rs1298550035	VUS (PM2,PP3,BP1)
3	49	(-)	Ovarian cancer	BRCA2	NM_000059.4	Exon 11	c.3751dupA	p.Thr1251AsnfsTer14	Insertion	rs397507683	Pathogenic (PVS1,PM2,PP3,PP5)
4	41	(+)	Multiple cancer history in the family	BRCA2	NM_000059.4	Exon 11	c.3751dupA	p.Thr1251AsnfsTer14	Insertion	rs397507683	Pathogenic (PVS1,PM2,PP3,PP5)
5	40	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 11	c.5578A>T	p.Lys1860Ter	SNV	rs431825332	Pathogenic (PVS1,PM2,PP5,BP4)
6	57	(+)	Ovarian cancer	BRCA2	NM_000059.4	Exon 11	c.6054_6058delTAACG	p.Ser2018ArgfsTer29	Deletion	**Novel**	Pathogenic (PVS1,PM2,PP3)
7	31	(-)	Breast Cancer	BRCA2	NM_000059.4	Exon 11	c.6562A>G	p.Lys2188Glu	SNV	rs1135401833	VUS (PM2)
8	33	(+)	Breast Cancer	BRCA2	NM_000059.4	Exon 19	c.8395delA	p.Arg2799AspfsTer22	Deletion	rs80359709	Pathogenic (PVS1,PM2,PP5)

Table 3. *BRCA1* and *BRCA2* copy number variations of our patients

Patient No	Age	Famiy History	Reason for application	Gene	Transcript	Exon	CNV type
1	43	(-)	Breast Cancer	BRCA1	NM_007300.4	3-5-6-7-8	Duplication
2	21	(+)	Family history of cancer	BRCA1	NM_007300.4	11	Deletion
3	18	(+)	Family history of cancer	BRCA1	NM_007300.4	11	Deletion
4	54	(-)	Ovarian cancer	BRCA1	NM_007300.4	11	Deletion
5	36	(+)	Family history of cancer	BRCA1	NM_007300.4	18-19	Deletion
6	65	(+)	Breast Cancer	BRCA1	NM_007300.4	18-19	Deletion
7	19	(+)	Family history of cancer	BRCA1	NM_007300.4	24	Deletion
Patient No	**Age**	**Famiy History**	**Reason for application**	**Gene**	**Transcript**	**Exon**	**CNV type**
1	23	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
2	56	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
3	53	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion
4	43	(+)	Family history of cancer	BRCA2	NM_000059.4	3	Deletion

individuals selected in line with the NCCN guidelines. The c.5266dupC (p.Gln1756Profs) variant in the BRCA1 gene, which is particularly common in the Ashkenazi population, was identified as the most common variant in this study at a rate of 5.47% [16]. This pathogenic BRCA1 variant was the most common (in three different patients) in our study too. Furthermore, in a large study of 1419 patients from Turkey in 2020, pathogenic variants were identified in (9.4%) 134 patients and likely pathogenic variants in (0.3%) five patients. BRCA1 variants were detected in 58 of these patients and BRCA2 variants were detected in 64 of them. Also, variants of uncertain significance were detected in (6.4%) 91 patients [17]. Additionally, less than breast and ovarian cancers, there is also an increased risk of developing other types of cancer at solid organs including prostate cancer, melanoma, and pancreatic cancer. Recent years, BRCA variant spectrum of pancreatic cancer has been determined both nationally and worldwide [18, 19].

In our study, the incidence of BRCA1 and BRCA2 variants in our cohort was 26.1%, which was higher than in previous studies conducted in Turkey. This result may be due to relatively lower number of patients in our study. It may have also resulted from the fact that a more well-selected group was tested.

When the distribution of variants on the gene was examined, it was seen that the variants in the BRCA1 gene were mostly (9/18, 50%) on the 10th exon. It was observed that the variants in the BRCA2 gene were mostly (6/9, 66%) on the 11th exon. When the literature, ClinVar and HGMD were examined, it was seen that these regions were hot-spot regions for these genes.

According to the literature, some BRCA1 and BRCA2 variants are common in certain populations. For example; 3 different variants (BRCA1 c.68_69delAG, BRCA1 c.5266dupC and BRCA2 c.5946delT) are seen in the majority of cases in Ashkenazi Jewish patients, which is also a testing criterion in the NCCN guidelines. When we look at the studies conducted in Turkish society, we see that the variants do not cluster but generally show a distribution. We also detected one novel variant each in BRCA1 and BRCA2

genes. The patient with a novel variant in the BRCA1 gene was a female diagnosed with breast cancer at 24 years old. Her family history was unremarkable. The detected BRCA1 variant (NM_007300.4:c.4246G>C: p.Ala1416Pro) was evaluated as uncertain clinical significance (VUS) according to the ACMG guidelines. The other patient, in whom we detected a novel variant in the BRCA2 gene, was admitted with the diagnosis of ovarian cancer at the age of 57. In the family story, her sister had ovarian cancer and her grandmother had a history of breast cancer. The detected BRCA2 variant (NM_000059.4:c.6054_6058del:p.Ser2018ArgfsTer29) was evaluated as pathogenic according to the ACMG guidelines.

Large deletions / duplications in the BRCA1 and BRCA2 genes differ between populations. It is estimated to be around 10 percent on average. Methods such as MLPA are used to detect large CNVs in the BRCA1 and BRCA2 genes. However, the multistep approach with CNV analysis followed by sequencing may be both expensive and time consuming. Recent developments with the NGS technology now allow simultaneous detection of CNVs and single nucleotide variations/small indels using different bioinformatic pipelines. We used Oncomine™ BRCA assay in combination with Ion Reporter ™ 5.16.0.3 software for this purpose. CNVs were found in %7.3 of cases in our cohort and all cases were confirmed by MLPA. Germani et. al (2018) reported %100 concordance between NGS results and MLPA/multiple amplicon quantification (MAQ) results using the same approach [20]. In another study, various assays analyzed with Sophia DDM platform and SeqNext software were compared with conventional methods [21]. Sensitivity of BRCA Tumor and BRCA HC assays analyzed with both Sophia DDM platform and SeqNext software were reported 100%. The specificity was highest (100%) for BRCA HC Assay-Sophia DDM platform combination and lowest (99.489%) for BRCA HC assay-SeqNext software combination. These data suggest that NGS-CNV detection algorithms show promise for a more efficient approach instead of multi-step testing. However, it is important to validate the assays and bioinformatic pipelines.

Conclusion

In this study, our experience of one year in our center is summarized in the light of scientific literature. We detected two novel variants in our cohort that were not previously described in the literature. For patients whose BRCA1 and BRCA2 variants could not be detected and who meet the NCCN hereditary breast and ovarian cancer syndrome criteria, multigene panels have been recommended to examine additional genetic causes. Further studies are needed in our country in order to evaluate the pathogenicity of BRCA1 and BRCA2 genes, especially variants of uncertain clinical significance.

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Original Article

Molecular Characterization Reveals the Importance and Diversity of Germline and Somatic RET Mutations in Cancer

Moleküler Karakterizasyonla Tespit Edilen Germline ve Somatik RET Mutasyonlarının Kanserdeki Önemi ve Çeşitliliği

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ABSTRACT

Aim: Many individuals die due to cancer, and both doctors and researchers work hard to offer accurate illness, diagnosis, and prognosis monitoring, as well as resistance prediction.
Methods: A liquid biopsy and hereditary cancer panels were performed on 25 patients to examine the importance, spectrum, and diversity of RET germline and somatic mutations. Most of the patients visited the clinic with the diagnosis of advanced resistant cancers or hereditary cancer (MEN2). Two groups were formed: the first group was germline (n=7, 28%), and the second was somatic (n=18, 72%). For somatic, Tier I-II-III variants; for germline, pathogenic, likely pathogenic, and VUS variants have been included in the study.
Results: The mean age was 54.64. There were significantly more female participants (n=14, 56%) than males (n=11, 44%). In the germline group, the most common mutation was 'RET:c.2410G>A'. Nine mutations were nonsense or frameshift in the somatic group, and the most common mutations were 'RET:c.2324delinsGAC' and 'RET:c.1784A>G'. Nonsense or frameshift RET variants showed a higher incidence in the somatic group.
Conclusion: To the best of our knowledge, this is the first research to concentrate on RET mutations in the context of genetic variability between germline and somatic variants. The current of the study results indicate that patients with solid tumors, particularly breast cancer, should undergo RET sequencing to evaluate clinical features and prognosis. Discoveries about the structure and functions of RET gene will lead to more clinically relevant treatment approaches for cancer patients and will play an essential role in improving individual risk prediction, treatment, and prognosis.

Keywords: Liquid biopsy, MEN2, RET

ÖZET

Amaç: Pek çok kişi kanser nedeniyle ölmekte. Hem doktorlar hem de araştırmacılar, doğru hastalık, teşhis ve prognoz takibinin yanı sıra direnç tahmini sunmak için çok çalışıyorlar.
Gereç ve Yöntem: RET germline ve somatik mutasyonların önemini, spektrumunu ve farkını incelemek için 25 hastaya likit biyopsi ve ailesel kanser paneli uygulandı. Hastaların çoğu ileri dirençli kanser ve / veya kalıtsal kanser (MEN2) tanısıyla kliniği ziyaret etti. Toplam iki grup oluşturuldu: birinci grup germline (n=7, %28) ve ikincisi somatik (n= 8, %72). Somatik için, Tier I-II-III varyantları ve germline için patojenik, muhtemelen patojenik ve VUS varyantları çalışmaya dahil edilmiştir.
Bulgular: Ortalama yaş 54.64 idi. Kadın katılımcılar (n=14, %56) erkeklerden (n=11, %44) önemli ölçüde daha fazla idi. Germline grubunda en yaygın mutasyon "RET: c.2410G>A" idi. Somatik grupta, dokuz mutasyon nonsense veya çerçeve kaymasıydı ve en yaygın mutasyonlar "RET: c.2324delinsGAC" ve "RET: c.1784A>G" idi. Nonsense veya çerçeve kayması RET varyantları, somatik grupta daha yüksek bir insidans gösterdi.

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Sonuç: Bildiğimiz kadarıyla bu, germline ve somatik varyantlar arasındaki genetik değişkenlik bağlamında RET mutasyonlarına odaklanan ilk araştırmadır. Mevcut çalışmanın sonuçları, solid tümörlü hastaların, özellikle meme kanserinin, klinik özellikleri ve prognozu değerlendirmek için RET sekansına tabi tutulması gerektiğini göstermektedir. RET geninin yapısı ve işlevleri hakkındaki keşifler, kanser hastaları için klinik olarak daha uygun tedavi yaklaşımlarına yol açacak ve bireysel risk tahmini, tedavisi ve prognozunun iyileştirilmesinde önemli bir rol oynayacaktır.

Anahtar Kelimeler: Likit biyopsi, MEN2, RET

Introduction

Receptor tyrosine kinases regulate cell development and differentiation. Some of them have been shown to behave as oncogenes in human malignancies. RET (rearranged during transfection) is a trans-membrane receptor tyrosine kinase that may act as both a growth factor receptor and an oncogenic protein. It is triggered by a complex that includes a soluble glial cell line-derived neurotrophic factor (GDNF) family ligand (GFL) and a glycosylphospha-tidylinositol-anchored co-receptor, GDNF family receptors a (GFRa) [1]. GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN) are four distinct GFLs that can bind to and selectively activate RET through their homologous co-receptors GFRa1–4. RET has multiple activities in diverse tissues as a signal transducer of four separate ligand/co-receptor complexes. It is required for the development of the enteric nervous system as well as the regulation of the development of sympathetic, parasympa-thetic, motor, and sensory neurons [2].

The RET protein is a receptor tyrosine kinase that seems to transduce growth and differentiation signals in a variety of developmental tissues, including neural crest-derived tissues. The protein comprises an extracellular domain containing a ligand-binding domain, a cadherin-like domain, and a cysteine-rich region proximal to the cell membrane. It includes one transmembrane domain and two tyrosine kinase subdomains, TK1 and TK2 [3].

Somatic and germline mutations in the same tumor suppressor gene are widely known, as detailed in Knudson's two-mutation paradigm [4]. Similarly, somatic and germline mutations in the RET protooncogene have been discovered in a number of hereditary and non-hereditary human disorders, including multiple endocrine neoplasia (MEN) 2A and 2B, papillary thyroid cancer, and other cancers [5].

Multiple endocrine neoplasia type 2 (MEN2), sometimes referred to as Sipple's syndrome, is linked with medullary thyroid carcinoma (MTC) and hyperplasia of thyroid C cells. It is an autosomal dominant genetic disorder caused by a mutation in the RET proto-oncogene on chromosome 10, which results in the development of two or more endocrine adenomas or hyperplasia in the same patient, either simultaneously or sequentially, and resulting in the clinical condition defined by hyperfunctioning glands [6].

MEN2 is classified clinically as MEN2A, MEN2B, and familial medullary thyroid cancer, with MEN2A being the most frequent subtype [1]. Medullary thyroid cancer (MTC), pheochromocytoma (PHEO), and hyper-parathyroidism are all characteristics of MEN2A. Additionally, a tiny percentage of people develop skin lichen amyloidosis or Hirschsprung's disease. MTC is often the initial symptom of this subtype, with a near-100 percent prevalence. When patients are hospitalized, the majority have already advanced to MTC or have lymph node metastases. MTC is the leading cause of mortality in people with MEN2A, and 50% of

patients are at risk of recurrence [7]. MTC or MEN2A, on the other hand, may manifest differently in family members. Specifically, fundamental lesions may be entirely or partially manifested, lesions in the affected endocrine glands may arise at various time intervals (which may be many years), and numerous endocrine glands may sometimes be affected and demonstrate concurrent start. At the moment, individuals with MEN2A who demonstrate MTC as an early symptom are often misdiagnosed [1].

Numerous malignancies are known to be oncogene-dependent: oncogene addiction has been shown in a variety of neoplasms [8]. Somatic RET gene fusions are known to be oncogenic drivers in a variety of tumor types and are seen in 1–2% of non-squamous NSCLC patients. Fusions of the RET gene result in the formation of chimeric, cytosolic proteins containing a constitutively active RET kinase domain [9]. The recent approval of numerous tumor-agnostic medications by the Food and Drug Administration has resulted in a paradigm shift in cancer therapy away from organ/histology-specific strategies and toward biomarker-guided treatments. Selpercatinib (LOXO-292), a novel RET-specific tyrosine kinase inhibitor, has shown exceptional effectiveness in cancers with RET fusions or mutations, most notably RET fusion-positive NSCLC and RET-mutated MTC [10].

Liquid biopsy techniques have been used to treat a variety of different forms of cancer in recent years. A liquid biopsy is utilized in tumors to determine the patient's recovery, prognosis, and even diagnosis. During apoptosis, tumor cells lose fragments of biomarkers. These materials' cellular components may be examined for genetic abnormalities. This less intrusive testing procedure provides a greater likelihood of a favorable outcome and a better probability of correct findings [11,12].

In this study, we performed a liquid biopsy and hereditary cancer panel on 25 patients to examine the importance, spectrum, and difference of germline and somatic RET mutations. Our data broadens the RET mutations and provides insights for the diversity and characteristics of somatic and germline RET mutations.

Materials and methods

Patients

Consent for the publication of the study and any additional related information was taken from the patients or their parents involved in the study. The Ethics Committee approved (2021-03/1072) the study at the University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital. Twenty-five patients visited the clinic with the diagnosis of advanced resistant cancers or hereditary cancer (MEN2). Clinical histories and molecular results were reviewed for all unrelated patients examined at the Department of Medical Genetics, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, and Department of Medical Genetics, University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Ankara, Turkey. The patients underwent the comprehensive liquid biopsy and hereditary cancer panel between January 2018 and December 2020 at the Ankara Central Genetic Laboratory (Turkey). In the study, a total of two groups were formed. The first group was germline (n=7, 28%) and the second was somatic (n=18, 72%).

DNA Panels and NGS

From the blood samples collected in EDTA tubes, the patients' genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen Inc., Hilden, Germany) by QIAcube (Qiagen Inc.,

Mississauga, ON, Canada). The DNA samples were quantified with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA).

Two different multigene panels have been used for liquid biopsy testing depending on the dates: ArcherDx Reveal ctDNA 28 Kit and Sophia Genetics 56 G Oncology. The Sophia Genetics 56G Oncology Solution was used at the center from 2018 to 2020, and the ArcherDx Reveal ctDNA 28 Kit has been used since 2020. The data were analyzed on the Archer Analysis Platform (ArcherDX, Inc., CO, USA) for the ArcherDx Reveal ctDNA 28 Kit and Sophia DDM software (Sophia Genetics, Saint-Sulp) for the Sophia Genetics 56G Oncology Solution.

For hereditary cancers, two different multigene panels were used depending on the dates: the Qiagen QIAseq Hereditary Custom Cancer Panel (from 2017 to 2018) and the Sophia Hereditary Cancer Solution Panel (since 2018). The sequencing was performed on an Illumina MiSeq system (Illumina Inc., San Diego, CA, USA). The data were analyzed using QIAGEN Clinical Insight (QCI™) Analyze software (Qiagen Inc., Hilden, Germany) for the Qiagen QIAseq Hereditary Custom Cancer Panel and with Sophia DDM software (Sophia Genetics, Saint-Sulp) for the Hereditary Cancer Solution (v1.1) panel. Visualization of the data was performed with IGV 2.7.2 (Broad Institute) software.

Interpretations, Descriptive Statistics & Graphics

In compliance with the recommendations issued by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, germline variants were categorized as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign [13]. Pathogenic, likely pathogenic, and strong VUS (supports clinical phenotype and no other responsible mutation detected) variations were included in the study. Somatic variants were categorized as tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants with unknown clinical significance; and tier IV, variants that are benign or likely benign, in compliance with the recommendations issued by the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists [14]. Tier I-II-III variations have been included in the study. Further, descriptive statistical calculations have been done, and the graphic has been prepared with Python 3.9.2 (IPython 7.19.0).

Results

The mean age was 54.64, with a minimum age of 35 and a maximum of 70. There were six patients below 50 years of age, and all of them were females. There were significantly more female participants (n=14, 56%) than males (n=11, 44%) (Table 1-2).

In the germline group, the mean age was 50.57, and all the mutations were missense and heterozygous. There were three pathogenic, two likely pathogenic, and two variant of uncertain significance (VUS) variants. The most common mutation was ‘RET:c.2410G>A’ (Table 1, Figure 1).

In the somatic group, the mean age was 56.22, and the variant fractions were between 0.1-10%. The majority of the patients have advanced-metastatic cancers. Nine mutations were nonsense or frameshift. The most common mutations detected were ‘RET:c.2324delinsGAC’ and ‘RET:c.1784 A>G’. The ‘RET:c.2324delinsGAC’ mutation has been observed seven times. (Figüre 1) In breast cancer, frameshift RET mutations were more predominant when compared with other groups (Table 2).

Table 1. RET germline mutations

Gender	Age	Indication	Gene	Mutation	Protein	Zygosity	Pathogenicity
F	53	colon	RET	c.1681A>T	p.Ser561Cys	heterozygous	Likely Pathogenic
F	35	MEN2	RET	c.224C>T	p. Thr75Met	heterozygous	Likely Pathogenic
F	41	MEN2	RET	c.785T>C	p.Val262Ala	heterozygous	VUS
M	67	MEN2	RET	c.341G>A	p.Arg114His	heterozygous	VUS
M	52	MEN2	RET	c.2370G>T	p.Leu790Phe	heterozygous	Pathogenic
F	56	MEN2	RET	c.2410G>A	p.Val804Met	heterozygous	Pathogenic
M	50	MEN2	RET	c.2410G>A	p.Val804Met	heterozygous	Pathogenic

Table 2. RET somatic mutations

Gender	Age	Indication	Gene	Mutation	Protein
F	53	advanced-metastatic	RET	c.1162G>A	p.Val388Ile
M	58	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
M	61	advanced-metastatic	RET	c.2071G>A	p.Gly691Ser
F	59	advanced-metastatic	RET	c.2372A>T	p.Tyr791Phe
M	66	advanced-metastatic	RET	c.1972C>T	p.His658Tyr
M	60	advanced-metastatic	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	48	breast	RET	c.1906A>C	p.Thr636Pro
M	62	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
M	51	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
F	37	breast	RET	c.2338_2339insC	p.Lys780Thrfs*64
F	69	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	46	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
M	57	advanced-metastatic	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	55	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	37	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
M	58	advanced-metastatic	RET	c.2341C>T	p. Gln781Ter
F	70	lung	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	65	advanced-metastatic	RET	c.2657G>A	p.Arg886Gln

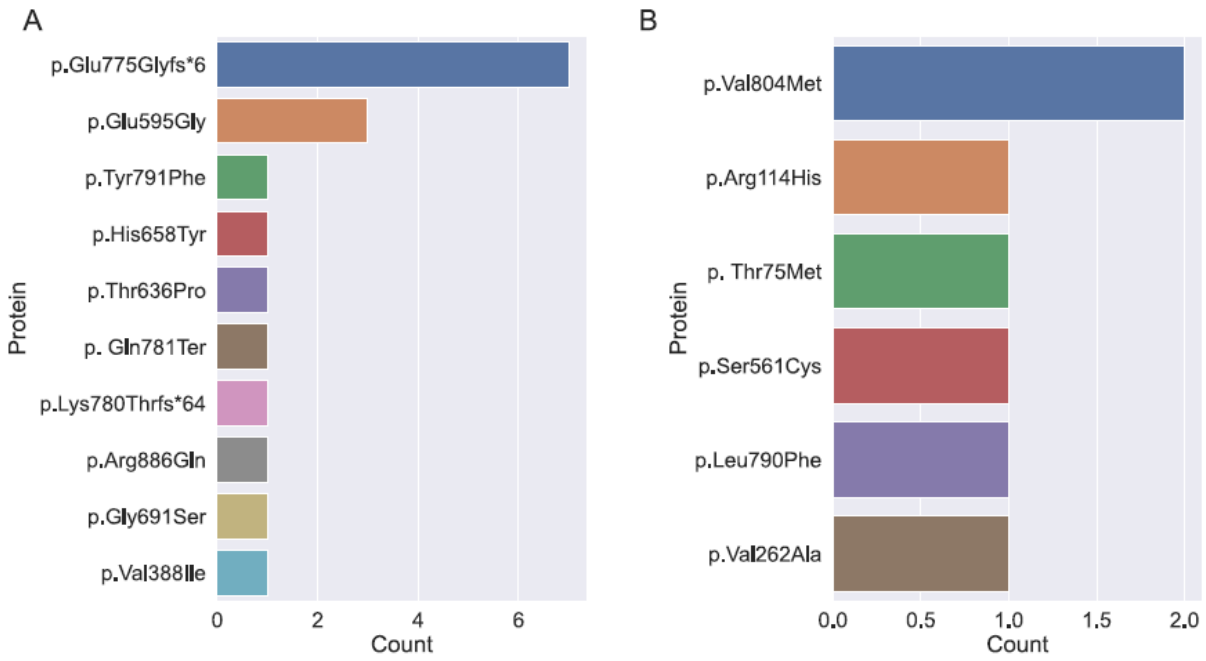

Figure 1. Somatic and germline RET mutations.
Bar plots showing the somatic (A) and germline (B) RET mutations in the study.

Discussion

Mutations in the RET gene result in various clinical symptoms and disease manifestations [2]. Based on RET's normal function, it is conceivable to identify various probable explanations for the disparate phenotypes. The signaling capability of various RET variants may be determined by subcellular location, substrate selectivity, turnover rate, percentage of activated RET, and genetic background. As a result, distinct types of clinical symptoms associated with RET may need treatment with different sorts of medications targeting specific domains of RET [2].

While germline mutations in codons 768 (exon 13), 804 (exon 14), and 891 (exon 15) are strongly related to MTC, they account for a small proportion of cases. These locations are located inside the domain of the intracellular tyrosine kinase. Exon 13 mutations are less prevalent in MEN2A/MTC (codons 790 and 791). Gatekeeper mutations in codon 804 have been found. Codon 804 mutation was found in two patients in the germline group in this study. (Figure 1) Changes at this location affect access to the RET ATP-binding domain, resulting in decreased sensitivity to some RET-targeting multi-kinase inhibitors [15]. Mutations in the intracellular TK2 domain are responsible for MEN2B-associated malignancies. A single 918 Met to Thr mutation in exon 16 accounts for almost 95% of MEN2B cases and is unique to this illness. Met 918 is a crucial component of the substrate recognition pocket found in the RET protein's tyrosine kinase catalytic core. Mutations arise as new (de novo) germline alterations in more than 50% of cases of MEN2B with codon 918 mutations. Another mutation, alanine to phenylalanine at codon 883 in exon 15, was discovered in some unrelated MEN2B relatives [16]. Dual (tandem) mutations in codons 804 and 806 or 804 and 904 may result in atypical MEN2B [17].

MEN2 RET mutations in the germline result in a gain of function. This contrasts with many other hereditary predispositions to neoplasia, which is caused by heritable "loss-of-function" mutations in tumor suppressor proteins. The functional restrictions imposed by such activating lesions are likely responsible for the rarity of RET mutations, a regulation that benefits molecular diagnostics in this condition [18].

Extensive research on large families demonstrates a clear genotype-phenotype link. MEN2B has a higher rate of morbidity and death than MEN2A. Survival is comparable between individuals with MEN2B and those with spontaneous MTC who had somatic RET mutations identical to the most prevalent germline mutations causing MEN2B. The genotype also affects the age at which MTC is first diagnosed and the result of thyroidectomy [19].

RET gene rearrangements are essential for solid tumors. In this study, nonsense and frameshift RET mutations were frequent in the somatic group, particularly breast cancer. 'RET, c.2324delinsGAC, p.Glu775Glyfs* 6' mutation was the most common. (Table 2, Figure 1) All the nonsense and frameshift RET mutations were on the 13th exon and in the kinase domain. The majority of the somatic group mutations were around the kinase domain. Most of the kinase domain RET mutations are oncogenic and associated with poor prognosis and drug resistance, particularly in thyroid cancers [20].

In contrast to the germline group, frameshift and kinase domain RET mutations were predominant in the somatic group. Many nonsense and frameshift RET mutations are also associated with gain of function according to databases (OncoKB), and they are likely oncogenic, unlike other genes. These mutations, particularly 'RET, c.2324delinsGAC, p.Glu775Glyfs*6', could be responsible for drug resistance,

progression, and metastasis. Further studies are needed to clarify the roles of these nonsense and frameshift RET mutations.

The current study's results indicate that patients with solid tumors, particularly advanced-metastatic cancers and breast cancer, should undergo RET sequencing to evaluate clinical features and prognosis. Discoveries about the structure and functions of RET gene will lead to more clinically relevant treatment approaches for cancer patients and will play an essential role in improving individual risk prediction, treatment, and prognosis.

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Original Article

Real-Life Analysis of Immunotherapy as the Second or Later Lines Treatment in Patients with Metastatic Non-Small Cell Lung Cancer

Metastatik Küçük Hücre Dışı Akciğer Kanseri Hastalarının İkinci veya İleri Sıra Tedavisinde İmmünoterapinin Gerçek Yaşam Analizi

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ABSTRACT

Background: Immunotherapy agents such as atezolizumab and nivolumab are appropriate option for non-small cell lung cancer (NSCLC) accounts in the absence of driver mutation, regardless of PDL-1 expression in second and later line setting. Herein we aimed to evaluate the efficacy and safety of immunotherapy for the second and later line settings in metastatic NSCLC patients as a single center experience.

Methods: Totally, 37 patients with metastatic NSCLC who received atezolizumab or nivolumab in the second or later lines were included. Clinicopathological features of patients and survival outcomes were analyzed. The safety profile and the factors that may predict survival were also evaluated.

Results: Twenty-nine (78.4%) of patients were men and 8 of patients (21.6%) were woman with median age of 61 years (range:42-80). Atezolizumab was preferred in 22 (59.5%) of these patients and nivolumab in 15 (40.5%) of them. Objective response rate was 35.1%. At a median follow up of 22.5 months, median progression-free survival (PFS) was 4.7 months, median overall survival (OS) was 24.1 months. Univariate analysis for PFS revealed that gender (p=0.03), age (p=0.005), the presence of brain metastasis (p=0.02), PDL-1 status >1% (p=0.035), ECOG PS (p=0.04) and the good response to frontline treatment (p=0.015) were found to be significant prognostic indicators. It also showed that the presence of brain metastasis (p=0.03), PDL-1 status >1% (p=0.027), good response to firstline treatment (p=0.022) and atezolizumab preference (p=0.018) were prognostic factors for OS.

Conclusion: Our real-life analysis indicated that atezolizumab and nivolumab improved survivals with good safety profile in second and later lines treatment of metastatic NSCLC patients.

Keywords: Non small cell lung cancer, atezolizumab, nivolumab, second or later line treatment

ÖZET

Amaç: Atezolizumab ve nivolumab, driver mutasyon yokluğunda, küçük hücre dışı akciğer kanserinin (KHDAK) ikinci ve sonraki basamak tedavisinde PDL-1 durumundan bağımsız olarak kullanılabilen iyi bir seçenektir. Burada, metastatik KHDAK'li hastalarda ikinci ve sonraki sıra tedavide immünoterapinin etkinliğini ve güvenliğini değerlendirmeyi tek merkez deneyimi olarak amaçladık.

Gereç ve yöntem: Çalışmaya, ikinci veya sonraki sıralarda atezolizumab veya nivolumab alan toplam 37 metastatik KHDAK hastası dahil edildi. Hastaların klinikopatolojik özellikleri ve sağkalım sonuçları analiz edildi. Güvenlik profili ve sağkalımı öngörebilecek faktörler değerlendirildi.

Bulgular: Hastaların 29'u (%78.4) erkek, 8'i (% 21.6) kadın, ortanca yaş 61 (aralık: 42-80) idi. Bu hastaların 22'sinde (%59.5) atezolizumab, 15'inde (% 40.5) nivolumab tercih edilmişdi. Objektif yanıt oranı %35.1 idi. Medyan 22.5 aylık takipte, medyan progresyonsuz sağkalım 4.7 (PSK) ay iken, medyan genel sağkalım (OS) 24.1 ay olarak bulundu. PFS için tek değişkenli analizde, cinsiyet (p=0.03), yaş (p=0.005), beyin metastazı varlığı (p=0.02), PDL-1 durumu >%1 (p=0.035), ECOG PS (p=0.04) ve ilk sıra tedaviye iyi yanıt varlığı (p=0.015) anlamlı prognostik göstergeler olarak bulundu. OS için ise, beyin metastazı varlığı (p=0.03), PDL-1 durumu >%1 (p=0.027), ilk sıra tedaviye iyi yanıt varlığı (p=0.022) ve atezolizumab tercihi (p=0.018) prognostik faktörler olarak bulundu.

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Sonuçlar: Gerçek hayat analizimiz, atezolizumab ve nivolumabın, metastatik KHDAK hastalarının ikinci ve sonraki basamak tedavilerinde iyi güvenlik profili ile sağkalımı iyileştirdiğini gösterdi.

Anahtar Kelimeler: Küçük hücre dışı akciğer kanseri, nivolumab, atezolizumab, ikinci ve sonraki sıra tedavi

Introduction

Lung cancer is the mostly diagnosed cancer worldwide and causes deaths approximately 1.7 million per year [1]. Non small cell lung cancer (NSCLC) is about 80% of lung cancers. Half of patients are diagnosed in the advanced setting, however survival rates are improving in recently years due to new treatment modalities [2]. Targeted therapies are appropriate option with presence of driver mutation e.g., epidermal growth factor receptor [EGFR]-mutant, anaplastic lymphoma kinase [ALK]-rearranged NSCLC. Nevertheless, in those with the lack of driver mutation immune check point inhibitors with or without chemotherapy is the best treatment option which has led to improvements in survival and quality of life [3]. Although immunotherapy is preferred at initial treatment setting, many patients are treated with frontline chemotherapy. For such patients regardless of PDL-1 expression status, anti-programmed cell death protein 1 (PD-1) or anti-programmed cell death ligand 1 (PDL-1) antibody is an appropriate option rather than single agent chemotherapy. Unlike atezolizumab and nivolumab, pembrolizumab is an option if the tumor PDL-1 has been identified in at least 1% of tumor cells [4-6]

Nivolumab, with the dose of 240 mg IV every two weeks, is an option for advanced NSCLC patients who progressed after platinum-based chemotherapy. In the phase III CheckMate 017 trial nivolumab compared with chemotherapy in squamous NSCLC and nivolumab improved overall survival (OS) with median 9.2 versus 6.0 months. PDL-1 status did not change the survival rates [7-9]. In the phase III CheckMate 057 trial, nivolumab was compared with docetaxel in advanced non squamous NSCLC, nivolumab also prolonged OS with median 12.2 versus 9.4 months. However, survival improvement was seen in PDL-1 positive tumors, which was similar between nivolumab and docetaxel for those with PDL-1-negative tumors [10,11].

Atezolizumab was approved for dose schedule 1200 mg IV every three weeks. In phase III OAK trial atezolizumab compared with docetaxel in advanced pretreated NSCLC with any PDL-1 and histologic status. Atezolizumab experienced improved OS, 13.8 versus 9.6 months regardless of histology. Atezolizumab versus docetaxel did not improve the PFS or response rates. Also higher PDL-1 status was related with greater OS results [12,13].

Pembrolizumab with approved dose of 200 mg every three weeks, was associated with better survival outcomes in pretreated advanced NSCLC whom at least 1 percent tumor cell PDL-1 expression. In Keynote 010 trial compared with chemotherapy OS difference was greater in patients with PDL-1 status >50% who received pembrolizumab, median 8.2 versus 16.9 months [14,15].

Despite clinical benefits, immuno-therapies can cause uniq side effects which is called immune-related adverse events. These side effect include dermatologic, gastro-intestinal, hepatic, endocrine, and other less common inflammatory events. Rarely fulminant and even fatal toxicities may occur with immune checkpoint inhibitors. In general, treatment of moderate or severe irAEs requires interruption of the checkpoint inhibitor and the use of glucocorticoid immunosuppression. Treatment of side effects are based on the

severity of the observed toxicity. Also the toxicity grade is important for the managment of side effects [16].

In the current study, we aimed to present the contribution and reliability of the use of immunotherapy to the survival of NSCLC patients who had received at least one frontline treatment, as a single center experience.

Methods:

Between 2015 and 2021, totally 37 patients with metastatic pretreated NSCLC who have received immunotherapy were included in this study. Patients who could not complete their treatment due to financial and non-illness reasons and those who died for reasons other than cancer, and the patients with ECOG PS 3 and 4 were excluded from data analysis. Patients' data were retrospectively obtained from patients charts with respect to age, number of metastatic sites, treatment choice, duration of treatment, PDL-1 status, survival outcomes and toxicities. The Local Ethics Committee of Istanbul Medipol University approved the study on June 2021 with E-10840098-772-02-2508 decision number.

PDL-1 Expression Assessment: The PDL-1 values of the patients were evaluated with the SP142 method in patients receiving atezolizumab and with the 22C3 method in patients receiving nivolumab.

Previous Treatment: As first-line therapy, 13 of 17 patients with adenocarcinoma histology received a paclitaxel-platinum regimen, while 4 received a pemetrexed-platinum regimen. Of 8 patients with squamous histology, 6 received paclitaxel-platinum and 2 received gemcitabine-platin chemotherapy regimen. Twelve patients using immunotherapy in the third-line received platinum-based doublet chemotherapy in the frontline setting, while they received gemcitabine-docetaxel chemo-therapy regimen in the second-line treatment.

Statistical analysis:

Statistical analyses were performed using SPSS 22.0 (SPSS Inc., Chicago, IL, USA). Survival analysis and curves were established according to the Kaplan-Meier method and compared by the long-rank test. PFS was defined as the time from diagnosis to the last follow-up and the time until relapse as being the time from diagnosis to the first evidence of relapse. In addition, OS was described as the time from diagnosis to the date of the patient's death or last known contact. Univariate and multivariate analysis of prognostic factors related to survival were performed by the Cox proportional hazards model. Multivariate p values were used to characterize the independence of these factors. The 95% confidence interval (CI) was used to quantify the relationship between survival time and each independent factor. All p values were two-sided in tests and p values less than or equal to 0.05 were accepted to be statistically significant.

Results:

Twenty-nine (78.4%) of patients were men and 8 of patients (21.6%) were woman with median age of 61 years (range:42-80). At the initial diagnosis, the majority of patients (64.9%) had advanced stage. Brain metastasis were detected in 15 patients (40.5%) at the initial diagnosis or during treatment. Histopathologically, most patients had adenocarcinoma (n=23, 62.2%). Eight patients had type 2 diabetes mellitus, ten patients had hypertension, in addition seven of patients had chronic obstructive lung disease. Patients and tumor characteristics are shown in Table 1.

PDL-1 positivity in adenocarcinoma histology was 52.2%, response rate to immunotherapy was 91.3%, while PDL-1 positivity in squamous cell histological subtype was 71.4% and response rate to immunotherapy was 85.7%. PDL-1 expression status was classified

Table 1. Patient and tumor characteristics

Characteristics	n	%
Total patients	37	
Age,years		
Median, range	61 (42-80)	
Gender		
Male	8	21.6
Female	29	78.4
Histopathological type		
Adenocarcinoma	23	62.2
Squamous cell carcinoma	13	35.1
Others	1	2.7
Initial clinical TNM stage		
Stage III	13	35.1
Stage IV	24	64.9
ECOG PS		
0	15	40.5
1	8	48.6
2	4	10.8
Tumor PD-L1 expression		
< 1%	21	63.6
1-49 %	8	24.2
>50%	4	12.1
Oncodriver mutation		
Absent	34	91.9
Present	3	8.1
Previous chemotherapy		
1	25	67.6
≥2	12	32.2
Choice of immunotherapy agent		
Nivolumab	15	40.5
Atezolizumab	22	59.5

Table 2: Response rates according to the RECIST 1.1

Response rate	n (%)
Complete response	0
Partial response	13 (35.1)
Stable disease	21 (56.8)
Progressive disease	16 (8.1)
Objective response rate (CR+PR)	13 (35.1)

*CR: Complete response, PR: Partial response,

as <1% in 21 (63.6%), 1-49% in 8 (24.2%) and >50% in 4 (12.1%) patients. There were three patients with presence of driver mutation as EGFR mutation who had adenocarcinoma histology. Therefore, they received targeted therapy in front-line setting. While 25 (67.6%) patients received immunotherapy in the second line setting, 12 patients (32.2%) received in the third and subsequent lines. Atezolizumab was preferred in 22 (59.5%) of these patients and nivolumab in 15 (40.5%) of them. The median cycles and duration of treatment were 5 (range: 2-24) and 3.7 months (range: 1.7-29.6).

Of the 22 patients who were treated with atezolizumab, 5 (20.8%) had partial response (PR) and 14 (58.3) had stable disease (SD). The PDL-1 expression level was measured in 22 of these patients, and the status >1% was measured in 10 of patients (54.2%). Twelve (54.5%) of the 22 patients used atezolizumab as a second line therapy.

Of the 15 patients who received nivolumab, 8 (53.3%) had PR and 7 (46.7%) had SD. The PDL-1 status was measured in 13 (86.7%) of these patients, and the PDL-1 status was >1% in six patients. Thirteen (86.7%) of 15 patients used nivolumab in second line setting.

Objective response rate (ORR) was 35.1% (Table 2). At a median follow up of 22.5 months, median PFS time was 4.7 months, while median OS time was 24.1 months (Figure1, Figure2). Brain metastasis occurred in 15 patients ongoing or pretreatment which were treated with radiotherapy. Cranial metastasis progressed only in 3 patients after radiotherapy. Pseudo-progression was seen in four patients (10.8%), hyper-progression did not occur in any patients.

Univariate analysis for PFS revealed that gender (p=0.03), age (p=0.005), the presence of brain metastasis (p=0.02), PDL-1 status >1% (p=0.035), ECOG PS (p=0.04) and the good response to frontline treatment (p=0.015) were found to be significant prognostic indicators. It also showed that the presence of brain metastasis (p=0.03), PDL-1 status >1% (p=0.027), good response to frontline treatment (p=0.022) and atezo-lizumab preference (p=0.018) were prognostic

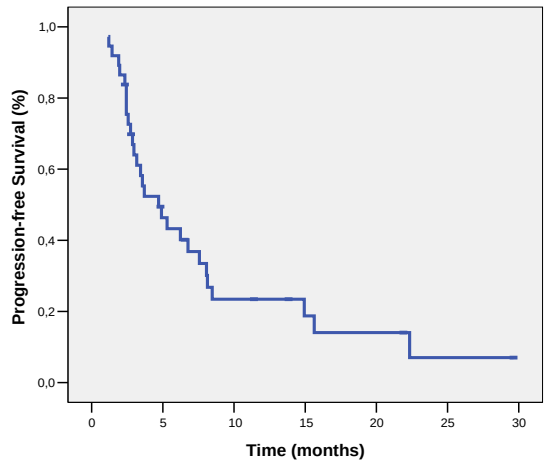

Figure 1: Median progression-free survival curve in patients with metastatic NSCLC

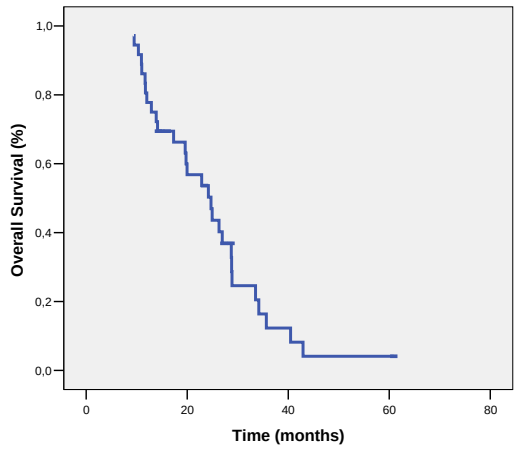

Figure 2: Overall survival curve in patients with metastatic NSCLC

factors for OS (Figure 3, Figure 4). Multivariate analysis indicated that good response to immunotherapy (HR:5.02, p=0.038) and good response to front line treatment (HR: 0.48, p=0.13), atezolizumab preference (HR:3.23, p=0.034) were significantly independent prognostic factors for OS. Figure 5 shows the OS which was significantly better for patients treated with atezolizumab compared with nivolumab arm. Moreover, gender (HR: 5.18, p = 0.0018), age (HR: 0.18, p = 0.003), ECOG PS (HR: 11.3, p =0.002), PDL status >1% (HR:0.32, p= 0.006) and good response to immunotherapy (HR: 0.26, p=0.002) were found to be significant independent prognostic indicators for PFS by multivariate analysis. Table 3 shows multivariate analysis for overall survival and progression-free survival.

The most common grade 3/4 adverse events regarding immunotherapy were pneumonitis in three patients (8.1%), colitis in one patient (2.7%). There was no need to discontinue the treatment due to side effects in neither nivolumab nor atezolizumab. While the dose was delayed in five (33%) of the nivolumab patients due to side effects, the dose was delayed in four (16.7%) of the atezolizumab patients. Moreover, rash (18.2%) and hypothyroidism (24.3%) were common immune-related grade 1-2 adverse events.

Discussion:

Initial treatment approach of advanced NSCLC patients is treating with immunotherapy in combination with platinum-based doublet chemotherapy in front line setting [17]. However, many patients will have treated with only platinum-based doublet chemotherapy. For such patients in the second line setting incorporation of immunotherapy is the preferred approach [3,12,14]. Nivolumab or atezolizumab are appropriate options regardless of tumor PDL-1 expression [4,5]. Pembrolizumab is an option for the tumors with at least >1% of PDL-1 status [6]. There is no data directly comparing these agents, so the choice among immunotherapies differs between centers by local practice and cost-effectiveness.

When the studies were evaluated, the median contribution of immunotherapy to overall survival for nivolumab, atezolizumab and pembrolizumab ranged from 9 to 13.8 months [9,12,15,18,20]. In our study, there were no patients who received pembrolizumab. Unlike, our real life experience with nivolumab

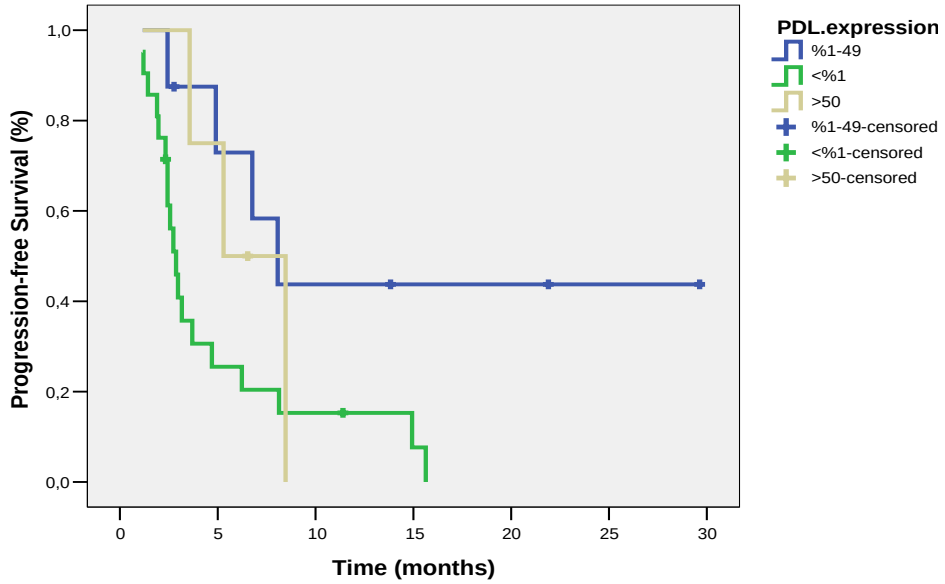

Figure 3: Progression Free Survival curves according to the PD-L1 expression

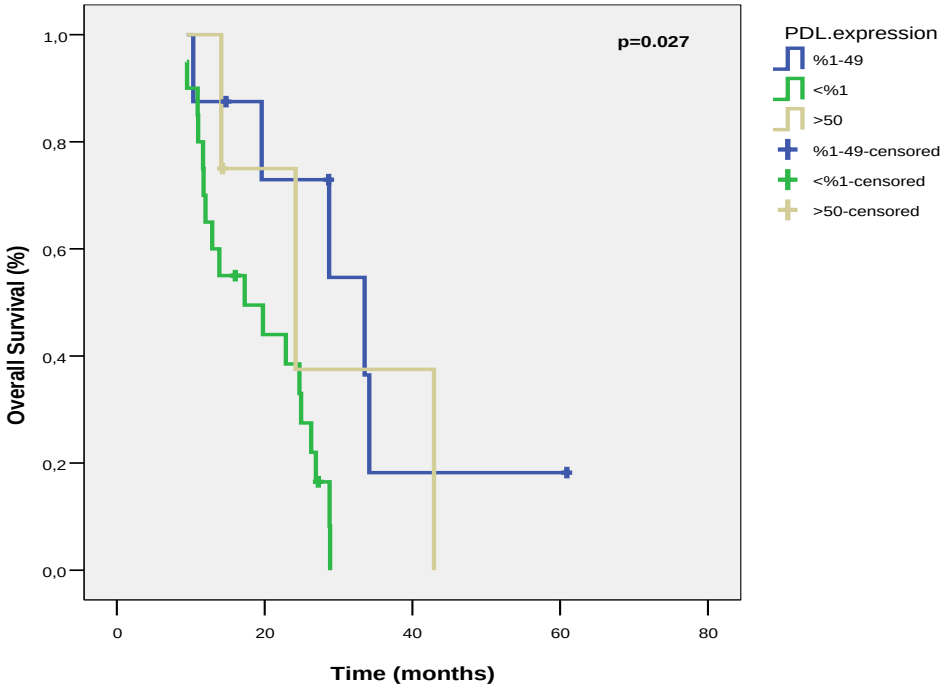

Figure 4: Overall survival curves according to the PD-L1 expression

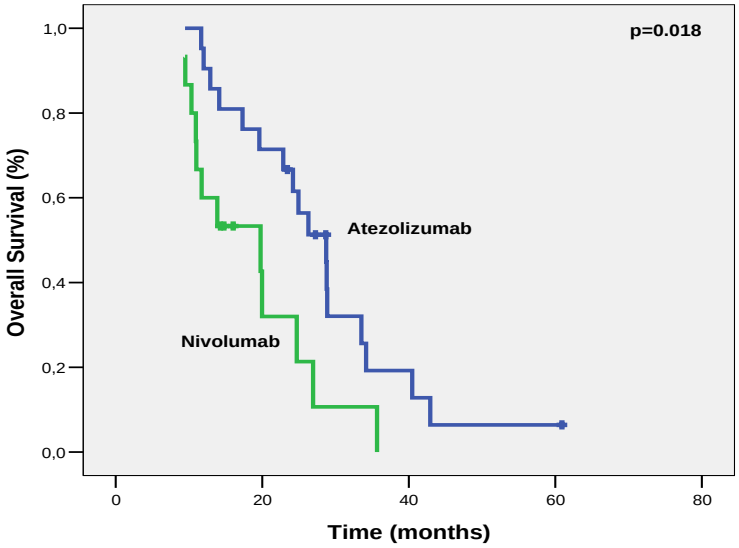

Figure 5: Overall survival curve for patients treated with atezolizumab compared with nivolumab

Table 3: Multivariate analysis for Overall survival and progression-free survival

Factor	X^2	p	HR	95% CI
Overall survival				
Response to immunotherapy	4.29	0.038	5.02	1.09-7.12
Presence of response to first-line chemotherapy	6.23	0.013	0.48	0.27-0.85
Immunotherapy type (Atezo vs Nivo)	4.47	0.034	3.23	1.09-4.09
PD-L1 expression (<1% vs 1-49% vs ≥50%)	3.23	0.072	0.45	0.19-1.07
Presence of brain metastasis	2.94	0.086	0.43	0.16-1.12
Progression-free survival				
Gender	6.98	0.0018	5.18	1.51-7.76
Age (<60 vs ≥60)	6.44	0.003	0.18	0.07-0.72
EGOG PS at the time of immunotherapy (0-1 vs 2)	8.71	0.002	11.3	2.09-19.1
PD-L1 expression (<1% vs 1-49% vs ≥50%)	7.65	0.006	0.32	0.24-0.72
Response to immunotherapy	9.81	0.002	0.26	0.11-0.60
Presence of brain metastasis	0.19	0.65	1.25	0.45-3.47
Presence of response to first-line chemotherapy	0.91	0.33	1.29	0.76-2.17

* HR: hazard ratio, CI: confidence interval, ECOG PS:

and atezolizumab is not similar to literature in terms of OS with median 24.1 months. This situation can be explained by the longer median follow-up period and the small sample size. On the other hand, median PFS interval was 4.7 months as similar to the literature [7,12,14]. One of the reasons for the longer overall survival in our study may be associated with the PDL-1 value >1% in 17 of 39 patients. In previous studies, the ORR with nivolumab was 19% in the squamous histological subtype, 20% in the non-squamous subgroup, while the ORR was 14% with atezolizumab [7,12,14]. However, in our study, ORR was 35.1%. Thus, our findings were not compatible with respect to OS and ORR [7,12,14,19]

In our study we showed that PDL-1 expression might differ according to the histologic type of the lung cancer. While PDL-1 positivity was 71.4% in the squamous cell subgroup, it was 52.2% in the group with adenocarcinoma. Similar studies in the literature determined the PDL-1 positivity in tumor cells was 56.2% in squamous cell carcinoma and 39.9% in adenocarcinoma [17]. One possible explanation for this difference may be that PDL-1 positivity is associated with smoking and squamous cell cancer is more frequently associated with smoking [20]. Previously studies showed the response to immunotherapy was worse in tumors with driver mutation [7,12,14]. In our study only three of patients have had driver mutation. Thus, no comment could be made.

Clinical trials in the second line setting included patients with stable brain metastasis [7,14]. As known brain metastasis is related with poor prognosis and in our cohort the number of patients with brain metastasis both at initial diagnosis and during treatment was 15 (4.5%). Although the survival contribution of immunotherapy is uncertain stereotactic radiosurgery was applied all of patients in our study.

An important point in drug preference is cost effectiveness. Under the conditions of our country, the use of nivolumab 240 mg every two weeks is a more expensive treatment compared to the use of atezolizumab 1200 mg every 3 weeks. Cost effectiveness is one of the reasons why atezolizumab is preferred more frequently in our center. In our center, 2 patients (5.4%) could not continue treatment due to financial reasons.

In fact there are no clear data to predict immunotherapy treatment response; however the factors found to be associated with longer PFS include; ECOG PS, smoking, liver metastases, lactate dehydrogenase (LDH), and neutrophil-to-lymphocyte ratio(NLR), absence of corticosteroid use and age > 50 years in the literature [23,24]. In our study, gender, age, the presence of brain metastasis, PDL-1 status >1%, ECOG PS and the good response to frontline treatment were found as prognostic factors in univariate analysis for PFS. As well multivariate analysis for PFS revealed that gender (HR: 5.18, p=0.0018), age (HR: 0.18, p=0.003), ECOG PS (HR:11.3, p=0.002), PDL-1 status >1% (HR: 0.32, p=0.006) and good response to immunotherapy (HR:0.26, p=0.002) were significant independent prognostic indicators. However, neither in Phase III CheckMate trials nor in OAK trial PDL-1 status was not found to be prognostic and/or predictive factor for the response [21,22]. Our results were thus not compatible with the literature [21,22].

Treatment-related adverse events of grade 3 or 4 were reported in 7% patients with nivolumab in CheckMate 017 and CheckMate 057 trial; 15% of patients who received atezolizumab in OAK trial [9]. Karak FE et al reported that all-grade immune-related adverse events were reported in around 18% of patients, and were mainly grades 2 and 3 [23]. In our series we reported grade 3-4 adverse events in four patients (10.3%) which was the pneumonitis in three patients, colitis in one patient. Any of treatment related

endocrine side effects were seen in eight patients (20.3%).

The small sample size and the retrospective design of our study could be considered as significant limitations and might have influenced these results. On the other hand, the long follow-up period and the management of immune-related side effects according to new guidelines were the positive aspects of our study. Therefore, we believe that our findings contribute to the literature, because we analyzed immunotherapy agents in both second and later lines, and in high PDL-1 positive patients with metastatic NSCLC as a single center experience.

In conclusion, our results indicate that both atezolizumab and nivolumab are active agents with good safety profile in second and later lines treatment for patients with metastatic NSCLC. Our real-life data is compatible with the results of previous clinical trials. However, the fact that the effectiveness is in a more PD-L1 positive group shows the need to identify predictive factors necessary to identify patients who will benefit from these drugs in the future.

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Original Article

A Comparison of the BEAM and BuCyE Conditioning Regimens for Autologous Stem Cell Transplantation in Lymphoma: A Single-Center Experience

Lenfomada Otolog Kök Hücre Transplantasyonu için BEAM ve BuCyE Hazırlama Rejimlerinin Karşılaştırması: Tek Merkez Deneyimi

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ABSTRACT

Introduction: High-dose chemotherapy together with autologous stem cell transplantation (ASCT) is a commonly used treatment modality in patients with relapsed/refractory Hodgkin's lymphoma (HL) or non-Hodgkin's lymphoma (NHL). The aim of this study was to investigate the efficacy and toxicity of BuCyE (busulfan, cyclophosphamide, and etoposide) and BEAM (carmustine, etoposide, cytarabine, and melphalan) conditioning regimens in patients with relapsed/refractory lymphoma scheduled for ASCT.

Methods: Between December 2018 and November 2019, 24 patients with relapsed or refractory HL (n=16) and NHL (n=8) who underwent ASCT following BEAM (n=12) and BuCyE (n=12) preparative regimens were analyzed retrospectively at Bone Marrow Transplantation Unit of Abdurrahman Yurtaslan Ankara Oncology Training and Research. The groups were compared in terms of patient characteristics, hematopoietic engraftment time, toxicity profiles, and progression free survival (PFS).

Results: No significant differences were detected between the groups with regard to age, gender distribution, ecog, sorror score, diagnosis, pre-ASCT stage (early/late), chemotherapy line, pre-ASCT response and pre-ASCT radiotherapy ($p>0.05$). The median number of infused CD34+ cells/kg, neutrophil and platelet engraftment statuses, duration of hospitalization, need for erythrocyte and platelet transfusion of BuCyE and BEAM groups were found to be similar ($p>0.05$). More patients in the BuCyE group developed mucositis and febrile neutropenia, but this difference was not statistically significant ($p>0.05$). At a median follow-up of 13 months(1–21 months) after ASCT, the median PFS could not be reached. No difference was found in PFS between regimes ($p = 0.68$).

Discussion and Conclusion: BuCyE followed by ASCT is an effective conditioning regimen in relapsed/refractory lymphoma patients. This regimen may be an important treatment option as a substitute for carmustine containing regimens. However, in the absence of prospective trials, it is difficult to suggest a conditioning regimen due to the low level of evidence. It is important to participate in ongoing clinical trials.

Keywords: Lymphoma, autologous stem cell transplantation, BuCyE, BEAM

ÖZET

Giriş ve Amaç: Otolog kök hücre transplantasyonu (OKHN) ile birlikte uygulanan yüksek doz kemoterapi, relaps/refrakter Hodgkin lenfoma (HL) veya Hodgkin dışı lenfoma (NHL) olan hastalarda yaygın olarak kullanılan bir tedavi yöntemidir. Bu çalışmanın amacı, OKHN planlanan relaps/refrakter lenfomalı hastalarda BuCyE (busulfan, siklofosfamid ve etoposit) ve BEAM (karmustin, etoposit, sitarabin ve melfalan) hazırlama rejimlerinin etkililiğini ve toksisitesini araştırmaktır.

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Yöntem ve Gereçler: Aralık 2018 ile Kasım 2019 arasında BEAM (n=12) ve BuCyE (n=12) hazırlık rejimleri ile OKHN yapılan nükseden veya dirençli HL (n=16) ve NHL (n=8) olan 24 hasta, Abdurrahman Yurtaslan Ankara Onkoloji Eğitim ve Araştırma Kemik İliği Nakli Ünitesi'nde incelendi. Gruplar hasta özellikleri, hematopoietik engraftman süresi, toksisite profilleri ve progresyonsuz sağkalım (PFS) açısından karşılaştırıldı.
Bulgular: Gruplar arasında yaş, cinsiyet dağılımı, ecog, sorror skoru, tanı, OKHN öncesi evre (erken/geç), kemoterapi sayısı, OKHN öncesi yanıt ve OKHN öncesi radyoterapi açısından anlamlı farklılık saptanmadı (p>0.05). BuCyE ve BEAM gruplarının ortalama infüze edilen CD34+ hücre/kg sayısı, nötrofil ve trombosit engraftman durumları, hastanede kalış süreleri, eritrosit ihtiyacı ve trombosit transfüzyonu benzer bulundu (p>0.05). BuCyE grubunda daha fazla hastada mukozit ve nötropenik ateş gelişti, ancak bu fark istatistiksel olarak anlamlı değildi (p>0.05). OKHN'den sonraki 13 aylık (1–21 ay) medyan takipte, medyan PFS'ye ulaşılamadı. Rejimler arasında PFS'de fark bulunmadı (p=0.68).
Tartışma ve Sonuç: BuCyE'yi takiben OKHN, relaps/refrakter lenfoma hastalarında etkili bir hazırlık rejimidir. Bu rejim, karmustin içeren rejimlerin yerine geçebilecek önemli bir tedavi seçeneği olabilir. Bununla birlikte, ileriye dönük çalışmaların yokluğunda, düşük düzeyde kanıt nedeniyle bir hazırlama rejimi önermek zordur. Devam eden klinik araştırmalara katılmak önemlidir.

Anahtar Kelimeler: Lenfoma, otolog kök hücre nakli, BuCyE, BEAM

Introduction

Most patients with Hodgkin lymphoma (HL) are cured with initial therapy. However, 5-10 % of the patients have a treatment-refractory disease and 10-30% will relapse following standard therapy. Although significant advances have been achieved in the treatment of non-Hodgkin's lymphoma (NHL), 40–60% of the patients still relapse or have a treatment-refractory disease [1].

Many randomized studies have shown significant improvements in progression-free survival (PFS) and event-free survival (EFS) with high-dose chemotherapy (HDC) and autologous stem cell transplantation (ASCT) in relapsed/refractory HL and NHL [1–3].

The most commonly used high-dose conditioning regimens in relapsed/refractory HL and NHL patients are BEAM (carmustine, etoposide, cytarabine, and melphalan), BEAC (carmustine, etoposide, cytarabine, and cyclophosphamide), CBV (cyclophosphamide, carmustine, etoposide), BuCyE (busulfan, cyclophosphamide, etoposide) and combination regimen with total body irradiation. BEAM is the most commonly preferred HDC regimen among these [4, 5]. The number of randomized studies comparing these regimens to date is quite low. Advances in conditioning regimens and supportive therapy have resulted in a reduction in transplant-related mortality. Although the search for a different regimen continues, recent supply and cost issues for carmustine have created an urgent need for alternative conditioning regimens [6].

The aim of this study was to investigate the efficacy and toxicity of BuCyE and BEAM conditioning regimens in patients with relapsed/refractory HL or NHL scheduled for ASCT.

Materials and methods

In this study, relapsed or refractory NHL or HL patients who received ASCT after salvage chemotherapy at Abdurrahman Yurtaslan Ankara Oncology Education and Research Bone Marrow Transplantation Unit between December 2018 and November 2019 were retrospectively analyzed. The patients with relapsed or refractory NHL and HL who had been diagnosed histopathologically were

Table 1. BuCyE and BEAM chemotherapy regimens

BuCyE protocol		BEAM protocol	
Busulfan (mg/kg)	16 (-7, -6, -5, -4. days)	Carmustine (mg/m^2)	200 (-7. day)
Cyclophosphamide (mg/kg)	120 (-3, -2. days)	Etoposide (mg/m^2)	200 (-6, -5, -4, -3. days)
Etoposide (mg/m^2)	400 (-3, -2. days)	Cytarabine (mg/m^2)	200 (-5, -4, -3, -2. days)
		Melphalan (mg/m^2)	140 (-2. day)

Table 2. Patient characteristics of all patients (n = 24)

Parameters	BEAM (n = 12)	BuCyE (n = 12)	P value
Age (median)	40 (20-59)	36,5 (27-65)	0,51
Gender (M/F)	9/3	10/2	1
ECOG (0/1)	5/7	4/8	1
Sorror Score (0/1-2)	10/2	11/1	0,6
Diagnosis (HL/NHL)	8/4	8/4	1
Disease type			
HL			
NS	5	5	
MC	2	2	
LR	1	-	
LD	-	1	
NHL			
DLBCL	3	3	
BL	1	1	
Pre-ASCT Disease Stage (I-II/ III-IV)	4/8	2/10	0,64
Chemotherapy Line (1-2/ ≥3)	7/5	5/7	0,41
Pre-ASCT Response (CR-PR/Progresyon)	11/1	11/1	1
RT (yes/no)	2/10	2/10	1

M: Male, F: Female, HL: Hodgkin's lymphoma, NS:Nodular Sclerosis , MC: Mixed Cellularity, LR: Lymphocyte Rich, LD: Lymphocyte Depleted, NHL: Non-Hodgkin's lymphoma, DLBCL: Diffuse large B cell lymphoma, BL: Burkitt lymphoma, ASCT: Autologous stem cell transplantation, CR: Complete remission, PR: Partial remission, RT: Radiotherapy.

accepted as suitable candidates for ASCT. All cases enrolled in the study were assessed in terms of chemosensitivity. The other inclusion criteria of the study were age <70 years, adequate heart, lung, liver, and kidney reserves, sufficient hematopoietic function, and Eastern Cooperative Oncology Group performance status of one or zero prior to ASCT. The study involved a total number of 24 patients with lymphoma scheduled for ASCT. Among these patients, 12 cases received BuCyE regimen, while BEAM was applied to 12 patients as preparative regimen prior to ASCT (Table 1). Successful neutrophil engraftment was accepted as an absolute neutrophil count of $\geq 1\times10^9$/L attained for one day, while platelet count $\geq 20\times10^9$/L without a need for platelet transfusion on the first consecutive three days after platelet engraftment was considered to be a successful platelet engraftment procedure. Treatment response was first evaluated one month after ASCT performed, then by 3-months intervals within the first 2 years. The groups were compared in terms of patient characteristics, hematopoietic engraftment time, toxicity profiles, and PFS. PFS was calculated as the time between the day of ASCT and data collection or exitus.

Table 3. Hospitalization process and findings after ASCT

Parameters	BEAM (n =12)	BuCyE (n = 12)	P value
Duration of diagnosis to ASCT (months) (median)	21 (4-214)	27,1 (9-91)	0,41
Diagnosis to transplant > = 24 months HL/NHL	4/1	7/1	
Diagnosis to transplant < 24 months HL/NHL	4/3	1/3	
Duration of Hospitalization (days)	22 (19-26)	22,5 (19-35)	0,29
Infused CD34 kg/cell (median)	9,8 (4,7-14)	6,59 (3,1-16,3)	0,14
Neutrophil engraftment (days) (median)	10(8-10)	10 (9-17)	0,09
HL (median)	10 (8-10)	10 (9-17)	
NHL (median)	10 (8-10)	10 (9-12)	
Platelet engraftment (days) (median)	11 (6-19)	10 (9-32)	0,35
HL (median)	12 (9-19)	10 (9-26)	
NHL (median)	10 (6-12)	12 (9-32)	
Need of ES transfusion(yes/no)	6/6	5/7	0,68
HL patients given ES transfusion	2	5	
NHL patients given ES transfusion	4	-	
Need of PLT transfusion (1-2/ ≥3)	8/4	6/6	0,4
HL patients given PLT transfusion (1-2/ ≥3)	4/4	4/4	
NHL patients given PLT transfusion (1-2/ ≥3)	4/-	2/2	
Mucositis (yes/no)	0/12	3/9	0,22
HL patients with mucositis	-	1	
NHL patients with mucositis	-	2	
Febrile Neutropenia (yes/no)	7/5	8/4	0,67
HL patients with febrile neutropenia	3	7	
NHL patients with febrile neutropenia	4	1	

ASCT: Autologous stem cell transplantation, HL: Hodgkin's lymphoma, NHL: Non-Hodgkin's lymphoma, ES: Erythrocyte suspension, PLT: Platelet.

Statistical analysis

Statistical analyses were performed with IBM SPSS (Version 26) software. Demographical data were summarized with descriptive statistics. Numerical variables were presented as median (minimum-maximum), categorical variables were presented as ratios. To compare groups, Mann Whitney U tests were used for numerical variables and Chi-square test was used for categorical variables. Kaplan-Meier survival analysis performed for PFS and log-rank test was applied to assess survival difference among groups. $P \leq 0.05$ was regarded as statistically significant.

Results

The median age of the patients was 38 (20-65). Of the patients, 33.3% had NHL and 66.7 % had HL. There were 19 (79.2%) male patients and 5 (20.8%) female patients. The median time between diagnosis and ASCT was 21 months (4-214) and 27.1 months (9-91) in BEAM and BuCyE groups, respectively. The characteristics of all patients are included in Table 2.

No significant differences were detected between the groups with regard to age, gender distribution, ecog, sorror score, diagnosis, pre-ASCT stage (early/late), chemotherapy line, pre-ASCT response and pre-ASCT radiotherapy ($p>0.05$) (Table 2). Median number of infused CD34+ cells/kg, neutrophil and platelet engraftment statuses, duration of hospitalization, need for erythrocyte and platelet transfusion of BuCyE and BEAM groups were found to be similar ($p>0.05$). More patients in the BuCyE group developed mucositis and febrile neutropenia, but this difference was not statistically significant ($p>0.05$) (Table 3).

At a median follow-up of 13 months (1–21 months) after ASCT, the median PFS could not be reached, and no difference was determined in PFS between the regimes ($p = 0.68$) (Figure 1).

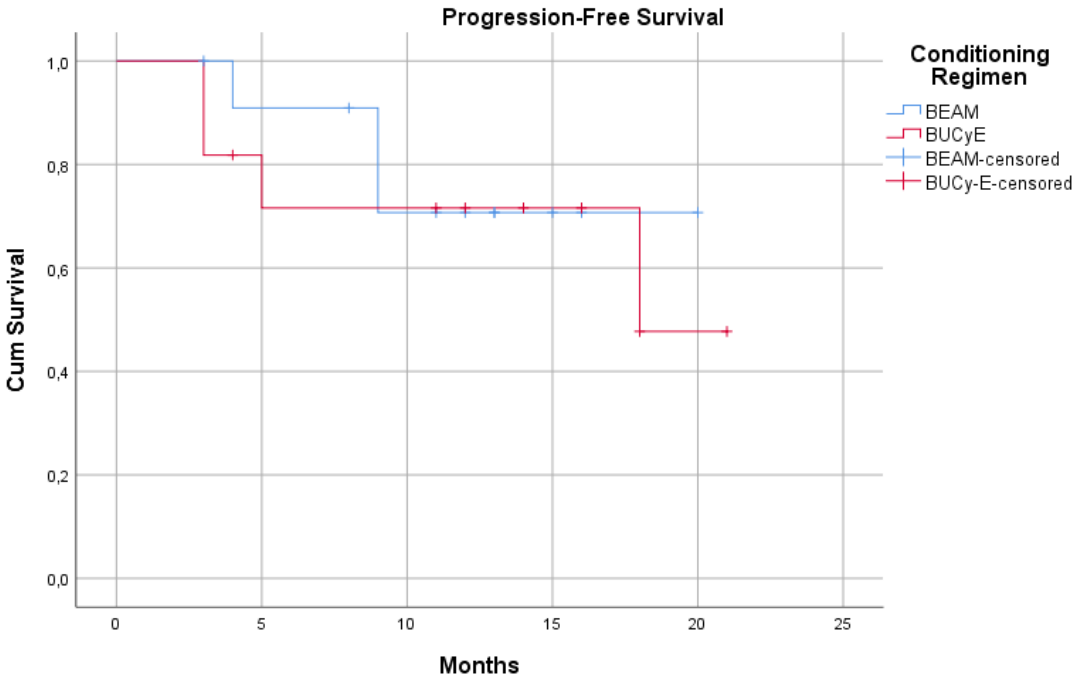

Figure 1. At a median follow-up of 13 months (1–21 months) after ASCT, the median PFS could not be reached and no difference was obtained in PFS between the regimes (p = 0.68).

Discussion

Despite the advances in modern chemotherapy, a significant proportion of patients with NHL or HL either never achieve remission or relapse early. For the vast majority of these patients, HDC followed by ASCT remains the best option for a long-lasting complete response. The most popular conditioning protocol for ASCT in lymphoma is BEAM.

Recent supply and cost issues, the high rate of mucositis requiring parenteral nutrition, and the high incidence of chronic interstitial pulmonary fibrosis for carmustine have created an urgent need for alternative conditioning regimens [6].

The resulting cost of carmustine, both drug-related and of managing toxicities, have spurred the development of novel regimens that replace this agent.

In several studies, bendamustine, thiotepa, fotemustine, lomustine, and mitoxantrone, have been examined as substitutions for carmustine in the BEAM regimen, resulting in similar or superior efficacy with a reduction in toxicity [7-10].

However, the lack of randomized trials using these agents and the fact that they include different study populations with differing proportions of histologies make it difficult to compare across studies.

Hanel M et al. conducted a study on 53 patients with HL or NHL who received high dose BuCyE conditioning regimen and investigated the efficacy and toxicity of BuCyE used as a preparative regimen prior to ASCT. In the evaluation of toxicities, mucositis (79%) and hepatic toxicity (15%) were found to be the most common non-hematological toxicities which were seen in 52 subjects, while three patients (5.8%) experienced severe veno-occlusive disease. In that study, the rate of treatment-related mortality was found as 3.8%. The authors concluded that BuCyE was an effective and well-tolerated conditioning regimen in patients with HL and NHL [5]. In our study,

none of the patients had veno-occlusive disease and in the BuCyE group, the rate of mucositis was 25% (n=3/12) and treatment-related mortality was not.

Singer S. et al. [11] retrospectively compared the BEAM and BuCyE for patients with relapsed NHL undergoing AHCT. After a median follow-up of 3.9 years for BEAM and 4.3 years for BuCyE from AHCT, PFS was found similar between the two conditioning regimens. In this study; it was reported that the number of CD34 infused was higher in the BuCyE group, the platelet engraftment time and hospital duration was shorter than in the BEAM group. In terms of adverse effects, mucositis was significantly more common in the BuCyE group, whereas sinusoidal obstruction syndrome was more common in the BEAM group.

Singer S. et al. [12] retrospectively compared the BEAM and BuCyE for patients with relapsed HL undergoing AHCT. They reported that the use of BEAM conditioning before AHSCT resulted in a statistically significant PFS, OS and lower relapse compared to BuCyE. In this study; it was reported that the number of CD34 infused was higher in the BuCyE group and the platelet engraftment time was shorter than in the BEAM group. They found the length of hospital stay was significantly shorter for the BEAM group and overall toxicities did not differ significantly between the two groups except for high rates of mucositis with BuCyE.

Berber et al. [13] compared 31 patients who received BuCyE and 11 patients who received BEAM in their study. No difference was obtained between the groups as regards the neutrophil and platelet engraftment duration and need for erythrocyte and platelet suspension during the transplantation. Also, mucositis, nausea, vomiting, diarrhea, infectious complications, and transplant-related mortality were found as similar. No statistically significant difference was determined between the groups as regards post-transplantation survival, total survival and EFS rates. As a result, BuCyE and BEAM were found as similar in terms of toxicity profile, and it was maintained that BuCyE could be an alternative preparation regimen. In our study, no difference was determined between both groups in terms of hospitalization duration, neutrophil and platelet engraftment duration, need for erythrocyte and platelet suspension, mucositis and febrile neutropenia, and thus it is similar to the study by Berber et al as regards the results.

In their study, Gunduz et al. [14] reported that in the patients given a BEAM (n=10) and BuCyE (n=10) preparation regimen, neutrophil and platelet engraftment duration, 100^{th} day remission state, hospitalization period, post-transplantation relapse and death, and need for total erythrocyte and platelet suspension were similar in both groups, but survival period is longer in the group receiving BEAM (55.25±15.29 vs. 12.12±4.02 months, $p = 0.02$). In our study, adverse effect profile, support treatment and hospitalization period were similar, and no difference was determined in PFS.

As a result, a small number of patients and a short follow-up time are insufficient to derive firm conclusions. However, a BuCyE conditioning regimen prior to ASCT was a well-tolerated and effective treatment for relapsed/refractory NHL and HL. This regimen may be an important treatment option as a substitute for carmustine containing regimens. Since, carmustine supply and cost issues urge for a search for alternative conditioning regimens.

However, in the absence of prospective trials, it is difficult to suggest a conditioning regimen due to the low level of evidence. It is important to participate in ongoing clinical trials.

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Original Article

Impact of Baseline Neutrophil-to-Lymphocyte Ratio on Outcomes of Glioblastoma Multiforme Patients Treated with Standart Concurrent Chemoradiotherapy

Başlangıç Nötrofil-Lenfosit Oranının Standart Eşzamanlı Kemoradyoterapi ile Tedavi Edilen Glioblastoma Multiforme Hastalarının Sonuçları Üzerine Etkisi

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ABSTRACT

Background: Glioblastoma multiforme (GBM) is the most common and miserable prognosis primary brain tumor in adults. Previously neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation has been demonstrated to have both strong predictive and prognostic value in different cancer types, which has rarely been addressed in GBM patients. The aim of this retrospective cohort study was to evaluatethe prognostic value of pretreatment NLR on survival outcomes of GBM patients who were underwent surgery/biopsy followed by definitive chemo-radiotherapy (CRT) and accessibility of a certain cut-off worth for NLR.

Material and Methods: This study was a hospital-based retrospective observational case-series study. This study was designed to identify 144 GBM patients with full pretreatment and treatment records that underwent surgery/biopsy followed by CRT from January 2007 to December 2011 in our clinics. Age, symptoms, laboratory results and treatment modalities of patients were recorded.

Results: The median follow-up time for the whole population was 15.1 (range 1.8-49.9) months, with 95 patients (84.8%) were death at the time of this analysis. NLR cut-off values of 4.3 (AUC:78.4; 95%CI: 64.8-92) for overall- (OS) and 4.1 (AUC:72.7; 95%CI:61-84.1) for local recurrence-free survival (LRFS) were identified, respectively, by using receiver operating curve analysis. Low NLR was associated with significantly longer median OS (23.2 vs. 12.7 months p=0.001), and LRFS (13.9 vs 9.6 months; p<0.001) as well as longer median both of which retained its independent significant association with survival outcomes in the multivariate analysis (p<0.001 for each).

Conclusion: In conclusion, pre-treatment low-NLR values associate with significantly longer OS and LRFS than those presenting with high-NLR. These findings suggest a novel strong and independent prognostic value for baseline NLR which is cheap, reproducible and easy to measure in routine clinical practice.

Keywords: Neutrophil, Lymphocyte, Glioblastoma Multiforme, survival

ÖZET

Giriş: Glioblastoma multiforme (GBM), yetişkinlerde en sık görülen ve en kötü prognoza sahip primer beyin tümörüdür. Daha önce sistemik inflamasyon belirteci olan nötrofil-lenfosit oranının (NLR), daha nadiren ele alınan GBM hastalarında da olmak üzere farklı kanser türlerinde hem güçlü prediktif hem de prognostik değere sahip olduğu gösterilmiştir. Bu çalışmanın amacı; tedavi öncesi NLR'nun cerrahi / biyopsi sonrası küratif kemoradyoterapi (KRT) alan GBM hastasının sağkalım sonuçları üzerindeki prognostik değerini ve NLR için belirli bir eşik değerin erişilebilirliğini değerlendirmekti.

Materyal-Metod: Bu çalışma, hastane temelli, retrospektif gözlemsel bir vaka serisi çalışmasıydı. Bu çalışma, kliniklerimizde Ocak 2007'den Aralık 2011'e kadar cerrahi/biyopsi ve ardından KRT uygulanan ön tedavi ve tedavi kayıtlarına sahip 144 GBM hastasını belirlemek için tasarlanmıştır. Hastaların yaş, semptom, laboratuvar sonuçları ve tedavi modaliteleri kaydedildi

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Bulgular: Tüm popülasyon için medyan takip süresi 15.1 (1.8-49.9) aydı ve bu analiz sırasında 95 hasta (%84.8) öldü. NLR cut-off değerleri curve analizi kullanılarak sırasıyla Genel sağkalım (OS) için 4.3 (EAA: 78.4; % 95 CI: 64.8-92) ve yerel rekürrenssiz sağkalım (LRFS) için 4,1 (AUC: 72.7; % 95 CI: 61-84.1) olarak tanımlanmıştır. Düşük NLR, istatistiksel olarak anlamlı şekilde daha uzun medyan OS (23.2'ye karşı 12.7 ay p = 0.001) ve LRFS (13.9'a karşı 9.6 ay; p <0.001) ve çok değişkenli analiz (her biri için p <0,001) ile de her iki parametre için daha uzun medyan sağkalım sonuçları ile ilişkilendirildi. Sonuç: Sonuç olarak, tedavi öncesi düşük NRL değerleri, yüksek NLR ile başvuranlara göre önemli ölçüde daha uzun OS ve LRFS ile ilişkilidir. Bu bulgular, başlangıç NLR'si için ucuz, tekrarlanabilir ve rutin klinik uygulamada ölçümü kolay yeni, güçlü ve bağımsız bir prognostik değer önerilebilir.

Anahtar kelimeler: Nötrofil, Lenfosit, Glioblastoma Multiforme, sağkalım

Introduction

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor in adults. According to the Stupp protocol the standard treatment incorporates maximal safe surgical resection, trailed by temozolomide chemotherapy concurrent with and adjuvant to focalized brain irradiation [1, 2]. However, the prognosis of such patients is extremely poor even after this aggressive treatment with a reported median and 2 years survival rates of only 14.6 months and 26.5%, respectively [3]. Therefore, GBM is invariably associated with inevitable recurrences and resultant deaths.

Albeit molecular pathology and genetic investigations on search of novel predictive and prognostic markers are advancing on a daily basis, yet there exists no universally accepted marker excluding the O6-methyl-guanine–DNA methyltransferase (MGMT) [4]. Traditional prognostic factors for GBM include the Karnofsky performance status, age, extent of resection, mental status, symptom duration at diagnosis, neurologic functionality, corticosteroid utilization, Mini-Mental State Examination score (MMSE), and radiotherapy dose [5,6]. Although combinations of these conventional factors effectively discriminate patients into groups with significantly differential outcomes they do not incorporate markers of systemic inflammation which may further be beneficial in prognostic sub-grouping of such patients.

Systemic inflammation has been demonstrated to promote local tumor progression and/or metastases by inducing angiogenesis and DNA damage repair system [7,8]. Accordingly, both the predictive and prognostic value of several biomarkers of systemic inflammation has been investigated in various tumor types [9-11]. High neutrophil-to-lymphocyte ratio (NLR) that is usually reflected by neutrophilia and lymphopenia, is one such biomarker that has been suggested to have a strong predictive and prognostic value in different cancer primaries [12-14]. Therefore, explore novel, convenient, practical and cheap biomarkers is necessary. Consequently, in this retrospective cohort study we planned to investigate the effect pretreatment NLR on survival outcomes of GBM patients who were treated with definitive CRT, and accessibility of a certain cut-off worth for NLR that may be utilized as a clinical indicator of survival outcomes in conjunction with promptly used traditional factors.

Methods and Materials:

We designed this retrospective study to identify 144 GBM patients with full pretreatment and treatment records that underwent surgery/biopsy followed by CRT from January 2007 to December 2011 in our clinics. To be eligible for the study, patients had to meet the following inclusion criteria: age ≥18 years, Karnofsky performance score (KPS) of 70 to 100, an adequate bone morrow reserve, hepatic and renal function. Additionally, patients had to have satisfactory preoperative and postoperative cranial magnetic resonance imaging (MRI) and surgery-CRT interval one month after surgery. Patients with any history of previous chemotherapy and/or cranial irradiation were

excluded. The study protocol was reviewed and approved by our Institutional Ethics Committee before collection of patients' data (project noKA 14/37).

Patient Records and Treatment

One month after surgery underwent a CT simulation for three-dimensional radiotherapy treatment planning (RTP). Gross tumor volume (GTV) was delineated on planning CT or its fusion with diagnostic CT/MRI. The RTP for eligible patients was based on GTV, which was restricted to primary tumors T1 contrast-enhancing tumor at MRI (without edema) on preoperative for patients who underwent biopsy or the surgical tumor bed plus any residual enhancing tumor that is seen on the planning scan in patients who underwent resection. The CTV is not defined. The PTV1 should include the GTV with a margin of 1 cm. However some cases may be used to adapt the PTV1 by excluding sensitive structures, such as the optic chiasm, chiasm, and brain stem. The PTV1 is treated with a dose of 40 Gy in 20 fractions. The PTV2 should include the GTV with a margin of 2 cm, and the PTV2 should be treated with 20 Gy in 10 fractions, for a total cumulative dose of 60 Gy. Concurrent chemotherapy consisted of TMZ at a daily dose of 75 mg/m^2 on 7 days a week from the first until the last day of RT. After a 4-week of break, patients received 4-6 cycles of adjuvant TMZ (150-200 mg/m^2 /d) for 5 days every 28 days. Prophylaxis against Pneumocystis carini with either pentamidine or trimethoprim-sulfamethoxazole was mandatory during concurrent RT and TMZ [2]. The available pre-CRT blood data of each patient was utilized to calculate neutrophil-to-lymphocyte ratio before using steroid.

Statistical Analysis

The primary endpoint was the impact of NLR on overall survival (OS) which was defined as the interval between the first day of CRT and death/last visit. Secondary objective was the identification of a particular cut-off value. For this purpose we used receiver operating characteristic (ROC) curve analysis. Survival curves were estimated according to the Kaplan-Meier method, and log-rank tests were used for univariate statistical comparisons. To evaluate the relationship between different variables and survival, a Cox proportional hazard model was used. All tests were two-tailed. A p-value ≤0.05 was considered significant.

Results

A total of 144 patients were reviewed and 112 patients who met the criteria were included in the study.. Patient characteristics are shown in Table 1. At a median follow-up of 15.1 (range 1.8-49.9) months for the whole study population, 95 patients (84.8%) were died. The median 2-years and 4-years OS rates were 15.1 months, 23.5% and 8.8% respectively.

The search for a special NLR cut-off by utilizing ROC analysis in the whole study population demonstrated the cut-off at 4.3 point (AUC:78.4; 95%CI=64.8-92; Sensitivity: 71.9%; Specificity: 69.6%), which was almost same with the cut-off of 4 and 4.73 previously defined one study and letter by Bambury and Alexious respectively [14,15]. Subsequently separated patients at this cut-off point into two groups: Low-NLR (L-NLR≤4.3) and high-NLR (H-NLR>4.3), the comparative survival analysis exhibited that the patients in L-NLR group had significantly longer OS (23.2 vs. 12.7 months; p=0.001) than their counterparts in H-NLR group. (Table 2, Figure 1). Consequently also detected for a special NLR cut-off value for locally recurrence free survival (LRFS) in our cases demonstrated the cut-off value at 4.1 value (AUC: 72.7 (95%CI: 61-84.1), and divided patients cut-off degree into two groups: Low-NLR (L-NLR≤4.1) and high-NLR (H-NLR>4.1). According to comparative LRFS analysis demonstrated that the patient in L-NLR group had paramount extended LRFS (13.9 vs. 9.6 months; p<0.001) than other group in H-NLR (Table 2, Figure 2).

We investigated the potential association between several prognostic factors. A univariate analysis was performed on the following factors: <50 age, sex,≥80 KPS, RTOG RPA classification, ≥3 months symptom duration, type of surgery performed

Table 1. Baseline patients and disease characteristics

Characteristic	N (%)
Age, y	
Median (range)	58 (32-69)
≤50	42 (37.5)
>50	70 (62.5)
Sex	
Male	76 (67.8)
Female	36 (32.2)
KPS	
70-80	39 (34.8)
90-100	73 (65.2)
RTOG RPA Class	
III	43(38.4)
IV	45 (40.2)
V	24 (21.4)
Extent of Surgery	
Complete resection	37 (41.9)
Partial resection	65 (58.0)
Biopsy	10 (0.10)
Symptom Duration	
<3 months	67 (59.8)
≥3 months	45 (40.2)
NLR for OS	
>4.3	46 (41.1)
≤4.3	66 (58.9)
NLR for LRFS	
>4.1	41 (36.6)
≤4.1	71 (63.4)

RTOG Radiation Therapy Oncology Group; KPS, Karnofsky Performance Score; RPA, recursive partitioning analysis; OS, overall survival; LRFS, locally recurrence free survival; NLR, neutrophil lymphocyte ratio

Table 2: Outcomes of overall and local recurrence free survival according to neutrophil-to- lymphocyte ratio

Survival	All patients	NLR ≤cut-off value*; #	NLR >cut-off value*; #	P-value
Overall survival*				
Median (mo, %95 CI)	15.1(12.9-17.3)	23.2(19.5-26.9)	12.7(9.7-15.7)	0.001
2 year (%)	23.5	43.2	13.9	
4 year (%)	8.8	20.6	0	
LRFS#				
Median (mo, %95 CI)	10.8 (9.3-12.3)	13.9 (11.2-16.6)	9.6 (8.3-10.9)	<0.001
2 year (%)	12.9	24.4	9.0	
4 year (%)	6.3	24.4	0	

NLR, neutrophil lymphocyte ratio; LRFS, Locally recurrence free survival * NLR ROC defined cut-off for overall survival ≤4.3, >4.3; #≤4.1, >4.1; NLR ROC defined cut-off for LRFS; mo, months; %95 CI, 95% confidence interval

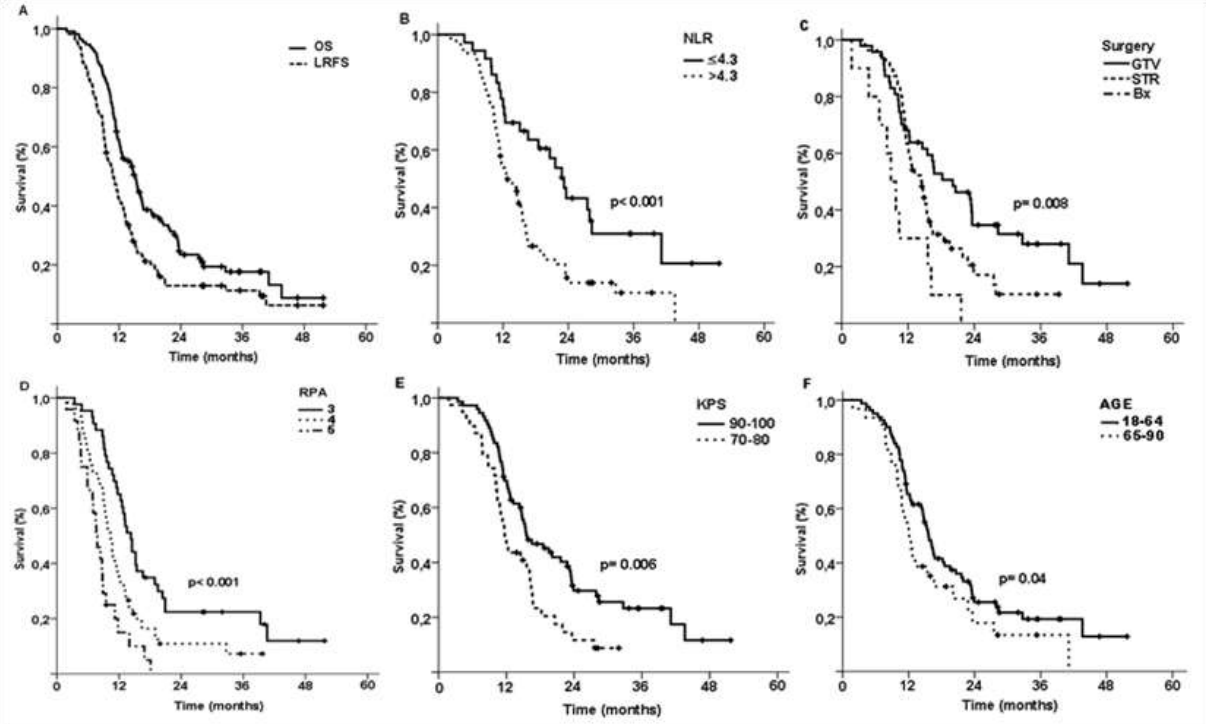

Figure 1:The whole population and comparative subgroup analysis for overall survival and locally recurrence free survival, A: Overall survival and locally recurrence free survival for whole study cohort, B. Neutrophil-to-lymphocyte ratio groups, C. Surgery type, D. Recursive partitioning analysis groups, E. Karnofsky performance score status, F. Age groups

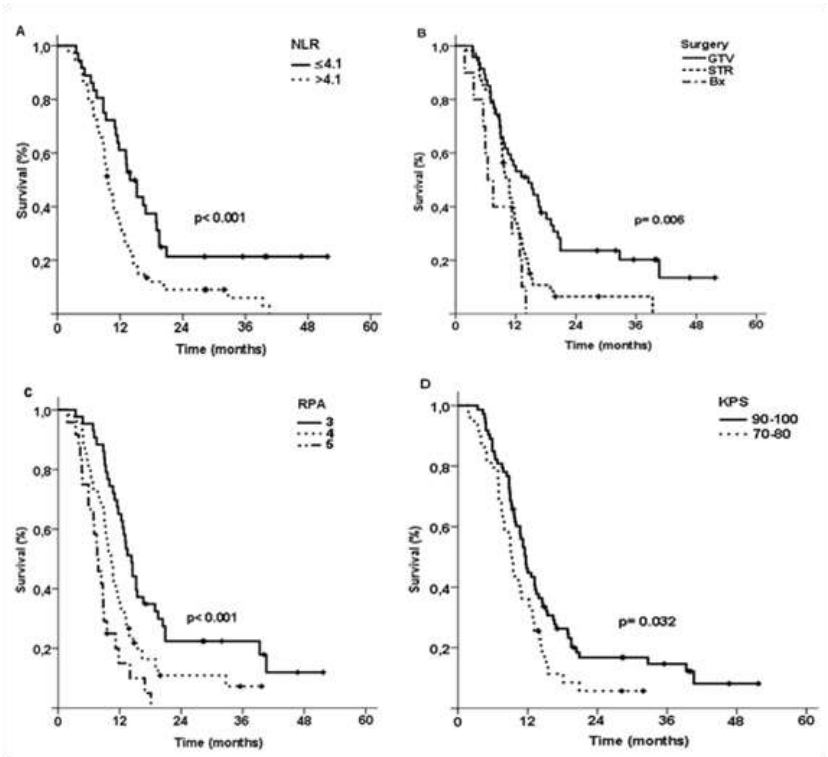

Figure 2: Outcomes of locally recurrence free survival analysis, A. Neutrophil-to-lymphocyte ratio groups, B. Surgery type, C. Recursive partitioning analysis groups, D. Karnofsky performance score status

Table 3: Results of uni and multi-variate analyses for overall survival and local recurrence free survival

Factor	OS Months (%95 CI)	Univariate P-value	Multivariate P-value	LRFS Months (%95 CI)	Univariate P-value	Multivariate P-value
Age, y						
≤50	15.6 (14.0-17.2)	0.03	0.04	11.7 (10.1-13.3)	0.249	
>50	12.1 (10.2-14.0)			9.7 (8.2-11.2)		
Sex						
Male	14.3 (11.1-17.5)	0.29		11.0 (9.8-12.2)	0.138	
Female	16.2 (11.6-20.8)			11.5 (8.0-15.0)		
KPS						
70-80	11.7 (10.1-13.3)	0.007	0.006	9.3 (8.3-10.3)	0.036	0.032
90-100	15.5 (13.0-18.0)			11.7 (10.5-12.9)		
RPA Class						
III	20.6 (12.3-28.9)	<0.001	<0.001	14.3 (12.5-16.1)	<0.001	<0.001
IV	14.5 (10.8-18.2)			10.4 (8.8-12.0)		
V	10.4 (9.2-11.6)			7.6 (5.9-9.3)		
Extent of Surgery						
Complete resection	20.1 (11.5-28.7)			14.5 (9.3-19.7)		
Partial resection	14.5 (12.0-17.0)	<0.001	0.008	10.7 (9.4-12.0)	0.007	0.006
Biopsy	8.9 (6.4-11.4)			6.4 (3.9-8.9)		
Symptom Duration						
<3 months	16.1 (14.5-17.7)	0.041	0.08	12.1 (10.5-13.7)	0.055	
≥3 months	12.1 (10.4-13.8)			9.6 (8.7-10.5)		
NLR						
≤*, #	23.2 (19.5-26.9)	<0.001	<0.001	13.9 (11.2-16.6)	<0.001	<0.001
>*, #	12.7 (9.7-15.7)			9.6 (8.3-10.9)		

RTOG Radiation Therapy Oncology Group; RPA, recursive partitioning analysis; KPS, Karnofsky Performance Score; NLR, neutrophil lymphocyte ratio; %95 CI, 95% confidence interval; * NLR ROC defined cut-off for overall survival ≤4.3, >4.3; #≤4.1, >4.1; NLR ROC defined cut-off for LRFS

(gross total resection) were significantly longer OS (p<0.05 for each), excluding the sex (p=0.29) (Table 3).

In multivariate analyses restricted to NLR (L-NLR vs. H-NLR), <50 age, ≥80 KPS, RTOG RPA classification, type of surgery performed (gross total resection), was the variable that retained its independent significance on association with OS time (p=<0.001, 0.04, 0.006, <0.001, 0.008 respectively), except for symptom duration (p=0.08) (Figure 1, Table 3).

We also investigated the potential association between aforementioned prognostic factors and LRFS. The univariate analysis was performed on the following factors: ≥80 KPS, RTOG RPA classification, type of surgery performed (gross total resection) were significantly longer LRFS (p=0.036, <0.001, 0.007 respectively), while other factors could not demonstrate any significance (p>0.05) (Table 3).

In addition multivariate analyses restricted to NLR (L-NLR vs. H-NLR), ≥80 KPS, RTOG RPA classification, type of surgery performed (gross total resection), was the variable that retained its independent significance on association with LRFS time (p=<0.001, 0.032, <0.001, 0.006 respectively) (Figure 1, Table 3).

Discussion

The results of present retrospective investigation suggested a prognostic value for pre-treatment NLR by demonstrating a strong association between the lower NLR ratio and superior LRFS (13.9 vs. 9.6 months; p<0.001) and OS durations (23.2 vs. 12.7 months; p=0.001)in newly diagnosed GBM patients who underwent surgery/biopsy followed by Stupp protocol, which may be used potential prognostic stratification in clinical. Given its relative cost-effectiveness in routine and cheaply be measured in any ordinary oncologic laboratory use, NLR is therefore a

suitable adjunct to other determinants of GBM prognosis.

Even if an important local control due to more than 85% of GBMs, still recurrence within the treatment field. GBMs are characterized by uncontrolled proliferation, diffuse infiltration of adjacent tissues, and revealed to identify prognostic factors in GBM patients enrolled in various clinical trials [17,18]. In the recently years, NLR is novel prognosticator marker allow the identification of inflammation and carcinogenesis which reflect disease biology, and numerous studies have suggested that an increased NLR is collaborated with poor survival of subject with various cancers. But there area limited number of studies in the literatureon this subject in GBM patients [15, 16,19].

In our study demonstrated that high neutrophil infiltration the progression of earlier can become. According to LRFS analysis demonstrated that the patient in H-NLR (NLR>4.1) group had paramount inferior LRFS than other group in L-NLR (NLR≤4.1) (9.6 vs 13.9 months; p<0.001). Although the exact mechanisms behind the role of increase NLR (elevated neutrophils count is associated by a decrease lymphocytes) in cancer worse prognosis effect is not to be explained with the design of our study, its reasonable to anticipate one of the possible mechanisms is association of H-NLR with inflammation, which is neutrophilia have been primary source of circulating VEGF, which has been shown to have a crucial role tumour- related angiogenesis and thus has a near relationship with vascular invasion and metastasis [20], and an inflammatory response inhibits the immune system by depressing the cytolytic activity of immune cell, and secrete tumor growth promoting factor [21-27]. On the other hands, lymphopenia is dependent to the immune escape of tumour cells from tumour-infiltrating lymphocytes (TIL) [28,29], and thus both increase infiltration of tumors and systemic is lymphocytes associated with better response to cytotoxic treatment and prognosis in cancer [30,31]. Therefore elevated neutrophils count is associated by a decrease lymphocytes means that may be immune deficiency of the patient. For this reason, due to with patient H-NRL is not sufficiency immune response, tumor may be progression earlier. Our study demonstrated that the patient in H-NLR group had paramount inferior LRFS than other group in L-NLR. For these reasons in our study thought high neutrophil infiltration that the progression of earlier can become.

Another important results of our study that patients with L-NLR (NLR≤4.3) had significantly longer median (23.2 vs 12.7; p=0.001), 2-year (43.2% vs. 13.9%) and 4-years (20.6% vs 0%) OS rates compared to those with H-NLR (NLR>4.3), suggesting a strong prognostic worth for pre-treatment NLR. In accordance with the first study performed by Bamburay et al. suggested that evaluable NLR>4 presented a worse independent prognostic factor in 84 GBM patients [15]. But Bamburay et al. study had been only 27% patient performed gross totally resection, 24% patients ECOG 2 and 58% patients were able to delivered concurrent chemoradiotherapy plus ≥2 cycles consol-idation TMZ. At the same time author only analyzed overall survival. Different in our cohort, 41.9% patients perform gross totally resection, all of them patients KPS ≥80 and were able to delivered concurrent chemo-radiotherapy plus ≥2 cycles consoli-dation TMZ. The other letter study show that pre-treatment NLR in 51 GBM patients with longer OS (NLR ratio <4.73; p=0.01). In multivariate analyses NLR ratio and extent tumor resection were recognized independent prognostic factor (p=0.01, p=0.025 respect-ively) [16]. Alike ours, these results suggested that L-NLR patients had higher local control than H-NLR therefore reflected longer survival in our study L-NLR patients, or body defenses system and immunity stronger L-NLR patients than H-NLR patients.

In GBM literature conventional prognostic factors were analyzed including age, duration time of diagnosis and surgery, sex, KPS, RTOG RPA classification, type extent of surgery resection. According to uni- and

multivariate analysis except for the sex and symptom duration; the other factors namely ≤50, KPS>80, RTOG RPA class III, gross total resection detected OS (p=0.03, 0.007, <0.001, 0.041 and p= 0.004, 0.006, <0.001, 0.008 respectively) longer than respective counterparts. However, uni- and multivariate analysis for LRFS detected KPS>80, RTOG RPA class III, gross total resection significant longer than respective counterparts (p=0.036, <0.001, 0.007 respectively), other prognostic factor was not significant (p>0.05). During the analysis MGMT was not routinely used by our pathology, therefore we didn't analyze the potential effects of the molecular marker MGMT and NLR value correlation.

Our present study has considerable limitations. First, as with any retrospective single-institution study, unpredictable biases may have influenced our results. Second, we did not have data on MGMT methylation status in our study population. But Han et al. study demonstrated NLR levels did not correlate with O-methylguanine-DNA methyltransferase (MGMT) promoter methylation status, they suggesting that these two prognostic factors may influence clinical outcome via different pathways and mechanisms [32]. Third, our sample size probably small, and we did not analyze possible predictive influence of NLR in GBM patients. Fourth; we did not investigate other potential prognostic factors ie: CPR, platelet to lymphocyte ratio, VEGF, MVP, MMP. Finally, our study warrants further confirmation in large prospective sample cohort studies with a definitive NLR cutoff value.

Conclusions

In conclusion, our study demonstrated that GBM patients presenting pre-treatment L-NLR associated with better immunity status, better response to cytotoxic treatment, prognosis, and so have significantly increased median, long-term survival rates and LRFS than those presenting with H-NLR. Such patients may be beneficial for the selection of individuals requiring more intense treatment, and may lead to a review of our current approaches for treating GBM patients with H-NLR. These findings suggest a novel, strong and independent prognosticator value for baseline NLR, which can easily, routine and cheaply measurable in any ordinary oncologic laboratory.

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Original Article

Salvage Treatment Options for Glioblastoma: Is Re-Operation Beneficial in Early Recurrence?

Glioblastomda Kurtarma Tedavi Seçenekleri: Erken Nükslerde Re-Operasyon Katkı Sağlar mı?

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ABSTRACT

Introduction: We aimed to investigate salvage treatment options for glioblastoma and to explore the role of surgery in early progression.

Methods: The study was designed as a retrospective review of 73 recurrent glioblastoma patients treated between July 2011 and March 2016. Patients were divided into two groups according to time of progression and re-treatments were analyzed for each. Early and late progressions were defined as progression before and after completion of the standard treatment package (≤9 months versus >9 months). Survival analysis were made with Kaplan-Meier method. Survival time comparisons between groups were made with Log-Rank test. Effect of variables on survival times were evaluated with Cox-Regression Analysis.

Results: Median overall survival time from the first diagnosis was 20 months (95% CI 17.0-22.9) and 2-year survival rate was 32.9%. Median time to progression was 10 (1-42) months. Median post progression survival (PPS) time was 8 months (95% CI 6.2-9.8). In multivariable analysis, we found early progression (9 months or less, $p<0.001$) and the use of supportive care after progression ($p<0.001$) as negative prognostic factors for PPS. In late progression, re-operation provided higher rates of PPS than systemic therapy (median 27 vs 10 months, p: 0.005) and supportive care (median 27 vs 3 months, $p<0.001$). However, no significant difference was found between reoperation and supportive care in case of early progression (median 3 vs 1 months, $p=0.143$).

Discussion and Conclusion: Progression is inevitable after standard treatment of glioblastoma. Survival after relapse is considered to be shorter than a year and appropriate patient selection is crucial when deciding on re-treatments. Survival rates of patients with progression earlier than 9 months are lower, and reoperation may not be an ideal option for this group.

Keywords: glioblastoma, reoperation, re-irradiation, temozolomide, bevacizumab

ÖZET

Giriş ve Amaç: Glioblastom, kötü seyirli, erişkinlerde en sık görülen primer beyin tümörüdür. Standart tedavi sonrası hastaların hemen hepsinde progresyon gelişmektedir. Progresyonda re-operasyon, sistemik tedaviler ve re-irradyasyon uygulanabilmektedir. Erken progrese olanların prognozu geç progrese olanlara göre daha kötüdür. Bu çalışmada progresyon zamanına göre kurtarma tedavi seçeneklerinin irdelenmesi amaçlandı.

Yöntem ve Gereçler: Temmuz 2011-Mart 2016 arasında tedavi edilmiş 73 nüks glioblastom tanılı hasta restospektif olarak değerlendirildi. Standart tedavi programı tamamlanmadan önce (≤9 ay) progrese olanlar 'ERKEN', tamamlandıktan sonra (>9 ay) progrese olanlar 'GEÇ' progresyon olarak tanımlandı.

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Her iki grup için kurtarma tedaviler irdelendi. Sağkalım analizleri için Kaplan-Meier metodu kullanıldı. Tek değişkenli analizlerde log rank, çok değişkenli analizde cox-regresyon testi kullanıldı.
Bulgular: İlk tanıdan itibaren genel sağkalım 20 ay (%95 CI 17.0-22.9), 2 yıllık genel sağkalım %32.9 olarak bulundu. Medyan progresyon zamanı 10 (1-42) aydı. Progresyon sonrası genel sağkalım 8 ay (%95 CI 6.2-9.8) olarak saptandı. Çok değişkenli analizde, 9 aydan erken progresyon ($p<0.001$) ve destek tedavi ($p<0.001$) sağkalımı negatif yönde etkileyen faktörler olarak bulundu. Geç progresyonda cerrahinin, sistemik tedaviden (medyan 27'ye karşı 10 ay, p: 0.005) ve destek tedavisinden (medyan 27'ye karşı 3 ay, $p<0.001$) daha iyi sağkalım sağladığı gözlendi. Erken progresyonda ise re-operasyon ve destek tedavisi arasında fark saptanmadı (medyan 3'e karşı 1 ay, p: 0.143).
Tartışma ve Sonuç: Glioblastomda standart tedaviler sonrası progresyon kaçınılmazdır. Progresyon sonrası sağkalım 1 yıldan kısadır ve kurtarma tedavi seçenekleri için uygun hasta seçimi önemlidir. Erken progresyon gösteren hastaların sağkalımı düşüktür ve re-operasyon bu hastalar için uygun olmayabilir.

Anahtar Kelimeler: glioblastoma, re-operasyon, re-irradyasyon, temozolomid, bevasizumab

Introduction

Glioblastoma, also known as Glioblastoma multiforme (GBM) is the most common central nervous system tumor in adults. At present, the standard treatment of GBM is maximal safe resection followed by 60 Gy conventional radiotherapy concurrently with temozolomide (TMZ) and adjuvant maintenance TMZ. Despite the current protocol, most patients progress within a year and have a median survival of 14.6 months [1].

After progression, current treatment options are limited and often ineffective. Surgery is always a tried-out option in selected patients if the tumor is well-suited for surgery [2]. Numerous studies published assessing the role of chemotherapeutic agents and their combinations such as nitrosoureas, TMZ, bevacizumab (BVC), immunotherapeutics and targeted therapies [3,4]. Re-irradiation is also a safe option with the advances of technology. Particularly, fractionated stereotactic radiotherapy has been shown to be useful in progression [5]. However, improvements on survival are insufficient and standard therapy currently is lacking.

Various studies support the conclusion that progressive glioblastoma (pGBM) treatment should be determined on a patient-to-patient basis and careful consideration of factors such as clinical/performance status, age, and quality of life is vital to deciding on treatment [6]. The most discussed topic of pGBM management is the usefulness of re-operation. Concerns about the additional morbidities of surgery, and short survival rates of the disease complicate the decision. Recently, a meta-analysis highlighted the timing of re-operation and showed that early re-intervention is associated with a higher risk of death than late re-intervention [7]. Patients with early progression may have an aggressive tumor moleculer profile, and this may be more important than the therapy itself. The progression time can have a significant impact on the treatment decision of pGBM.

The aim of the study is to demonstrate the prognostic role of the progression time and to investigate time-dependent salvage treatment options for pGBM.

Materials and Methods

Study Design

We retrospectively reviewed the medical records of patients treated for glioblastoma between July 2011 and March 2016. Inclusion criteria were as follows: (1) Patient had to have a radiologically proven progression after 1st treatment according to Response Assessment in Neuro-Oncology (RANO) Criteria [8], (2) patient had to attend follow-

up. Patients who could not be operated and only biopsied were excluded. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study protocol was approved by The Ethics Committee of Cerrahpaşa Medical Faculty (21.02.2020/30332) and all patients had written informed consent.

Initial treatment

All patients underwent maximal debulking surgery, followed by radiotherapy plus TMZ. Total resection was achieved on 35 (48%) patients, while 38 (52%) patients had subtotal resection. Radiotherapy (RT) was administered 60 Gy with conventional fractionation in 6 weeks. RT planning was done in a single-phase treatment plan with a 2-2.5 cm Clinical Target Volume (CTV) and 5mm Planning Target Volume (PTV) margin, including peritumoral edema. TMZ 75 mg/m^2 oral capsule was taken every day during RT. Eight patients (11%) received hypo-fractionated radiotherapy (40 Gy in 3 weeks) due to lower performance status and/or older age. Of these, four patients were administered with concomitant TMZ. After radiochemo-therapy, 6 cycles of adjuvant TMZ (150- 200 mg/m^2) were administered over a 28-day cycle for 5 days (1). Adjuvant TMZ was given 12 cycles in 2 (2.73%) patients and lower than 6 cycles (median 3) in 31 (42.5%) patients. It was stopped earlier due to hematological toxicity in four patients and rapid progression in 27 patients. After completion of treatment, the patients were followed up with a clinical examination and magnetic resonance imaging (MRI) every 2 months. Tumor progression was defined as the evidence of a new contrast-enhancing lesion or a ≥25% increase in size of a known contrast-enhancing lesion or remarkable increase T2/FLAIR abnormality on MRI.

Treatment after progression

Patients eligible for surgery after recurrence were operated as a first option, others were considered for chemotherapy firstly. TMZ was used as in the initial treatment dose, 150-200 mg/m^2, 5 days in every 4 weeks until progression. BVC was administered 10 mg/m^2 every two weeks. Reirradiation was used only in two patients with a hypofractionated scheme (6x5 Gy). Twenty patients were not eligible for any treatment and received only supportive care.

Data Collection

Data collection form includes age (at diagnosis), gender, tumor location, extent of resection, Karnofsky performance score (KPS), initial treatments, date of progressions, salvage treatments, date of death or last follow-up. The progression time was defined as the time from the first surgery to the first evidence of progression. Overall survival (OS) was defined as the time from the surgery to the last follow up or patient's death. Post progression survival (PPS) was defined as the time from the first evidence of progression to the last follow up or patient's death.

Statistical Analysis

All analyses were performed on SPSS package (SPSS 22.0 for Windows; SPSS Inc, Chicago, IL). Imbalances in categorical variables were tested using the Chi-square test. Survival analysis were made with Kaplan-Meier method. Differences were compared with Log-Rank test. Pairwise comparisons were made with Bonferroni correction method. Effect of variables on survival times evaluated with Cox-Regression Analysis with Backward Conditional method. $p<0.05$ values were accepted as statistically significant.

Results

A total of 147 patients were screened. We enrolled 73 eligible cases into our study,

Table 1. Patient and tumour characteristics

	Early progression, ≤9 month, n (%)	Late progression, >9 month, n (%)	All n (%)	p
Age				
<60	20 (64.5)	29 (69.0)	49 (67.1)	0.684
≥60	11 (35.5)	13 (31.0)	24 (32.9)	
Sex				
Male	15 (48.4)	24 (57.1)	39 (53.4)	0.459
Female	16 (51.6)	18 (42.9)	34 (46.6)	
Initial KPS				
≤70	12 (38.7)	9 (21.4)	21 (28.8)	0.107
>70	19 (61.3)	33 (78.6)	52 (71.2)	
Main Location				
Frontal	11 (35.5)	18 (42.9)	29 (39.7)	
Temporal	8 (25.8)	11 (26.2)	19 (26.0)	0.828
Parietal	8 (25.8)	10 (23.8)	18 (24.7)	
Occipital	4 (12.9)	3 (7.1)	7 (9.6)	
Extensiveness				
Single lobe	21 (67.7)	31 (73.8)	52 (71.2)	0.571
Multiple lobes	10 (32.3)	11 (26.2)	21 (28.8)	
First surgery type				
Total	13 (41.9)	22 (52.4)	35 (47.9)	0.377
Subtotal	18 (58.1)	20 (47.6)	38 (52.1)	
Treatment after progression				
Surgery	8 (25.8)	7 (16.6)	15 (20.6)	
Temozolomide	6 (19.4)	19 (45.2)	25 (34.2)	0.227
Bevacizumab	6 (19.4)	7 (16.7)	13 (17.8)	
Supportive care	11 (35.5)	9 (21.4)	20 (27.4)	

median age was 53 (24-79) years. Median overall survival time from the first diagnosis was 20 months (95% CI 17.0-22.9) and 2-year survival rate was 32.9%. Median time to progression was 10 (1-42) months. Median survival time after progression was 8 months (95% CI 6.2-9.8), 6- months and 1-year PPS rate was 58.9% and 30.1%, respectively. The patients were dichotomized into two groups according to the time of progression. Early progression, which was defined progression before completion of the standard treatment package (≤9 months), was seen in 31 patients. Late progression, which was defined progression after completion of the standard treatment package (>9 months), was detected in 42 patients. Two patients were still alive at the time of our review. Isocitrate dehydrogenase (IDH) mutations were positive in five patients, negative in 30 patients and missing in 38 patients. Five of the seven samples examined had methyl guanine methyl transferase (MGMT) promoter-methylation. MGMT status was missing in 66 patients. Demographic characteristics were given in Table 1.

After first progression, the most common treatment was TMZ in 25 (34%) patients. The second and third progressions were demonstrated radiologically with only 16 and 5 patients, respectively. Others experienced rapid clinical deterioration without an MRI diagnosis and did not receive tertiary treatments. (Table 2).

In univariable analysis, male patients had significantly higher PPS times than females (p=0.030). Patients with a score equal to or less than 70 KPS had a shorter PPS time than patients with a score higher than 70 KPS (p=0.088), and patients who had progression after 9 months had higher PPS times than those who did not (p<0.001). When we evaluated the type of treatment after relapse, the supportive care arm had a significantly shorter PPS than the others (p<0.001) (Table 3).

Table 2. Treatment methods after relapse

	First relapse (n=73)	Second relapse (n=16)	Third relapse (n=5)
Temozolomide	25 (34%)	2 (12%)	
Supportive care	20 (27%)	2 (12%)	1 (20%)
Surgery	15 (21%)	3 (19%)	
Only Surgery	8 (11%)		
Surgery + TMZ	3 (4%)		
Surgery + BVC	2 (3%)		
Surgery + RT + TMZ	2 (3%)		
Bevacizumab	13 (18%)		
BVC	11 (15%)	2 (12%)	2 (40%)
BVC + IRI	2 (3%)	6 (38%)	1 (20%)
PCV		1 (6%)	1 (20%)

TMZ:Temozolomide, BVC: Bevacizumab, RT: Radiotherapy, IRI:Irinotecan, PCV: Procarbazine, lomustine, vincristine.

Table 3. Survival rates after progression

	n	Median Survival (Month)	95% Confidence Interval		1- year Survival Rate (%)	p
			Lower	Upper		
Overall Survival after progression	73	8	6.2	9.8	30.1± 5.4	N/A
Age						
<60	49	9	7.0	10.1	32.7 ±6.7	0.642
≥60	24	6	1.2	10.8	25.0 ±8.8	
Gender						
Male	39	10	6.7	13.0	38.5 ± 7.8	0.030
Female	34	6	3.1	8.9	20.6 ± 6.9	
Initial Karnofsky Performance Status						
≤ 70	21	7	2.5	11.5	19 ± 8.6	0.088
> 70	52	9	5.9	12.1	34.6 ± 6.6	
Main Location						
Frontal	28	9	6.9	11.0	32.1 ± 8.8	
Temporal	20	7	6.0	8.0	26.3 ± 10.1	0.516
Parietal	18	9	3.7	14.3	35.3 ± 11.6	
Occipital	7	3	0.4	5.6	14.3 ± 13.2	
Extensiveness						
Single lobe	52	8	5.2	10.8	30.8 ± 6.4	0.658
Multipl lobes	21	9	5.7	12.3	28.6 ± 9.9	
First Surgery						
Total resection	35	9	6.7	11.3	31.4 ± 7.8	0.218
Subtotal resection	38	7	3.6	10.4	28.9 ± 7.4	
Progression time						
≤9 month	31	5	1.9	8.1	9.7 ± 5.3	<0.001
>9 month	42	10	6.0	14.0	45.2 ± 7.7	
Treatment After Relapse						
Temozolomide	24	9	6.6	11.4	36.0 ± 9.6	<0.001
Bevacizumab	13	9	6.9	11.1	30.8 ± 12.8	
Surgery ± adjuvant	15	13	1.6	24.4	53.3 ± 12.9	
Supportive care	20	2	0.7	3.3	5 ± 4.9	

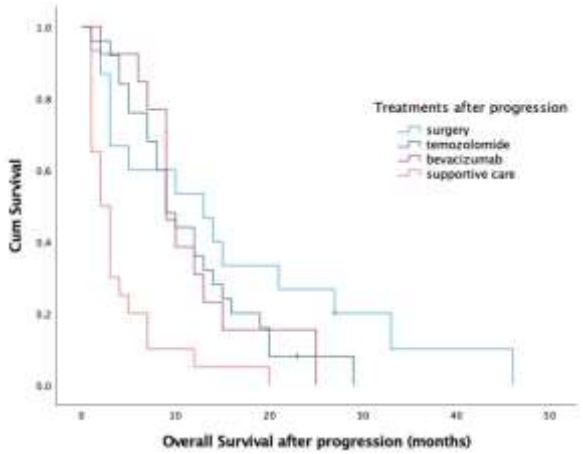


Figure 1. Survival curves of treatment groups after progression

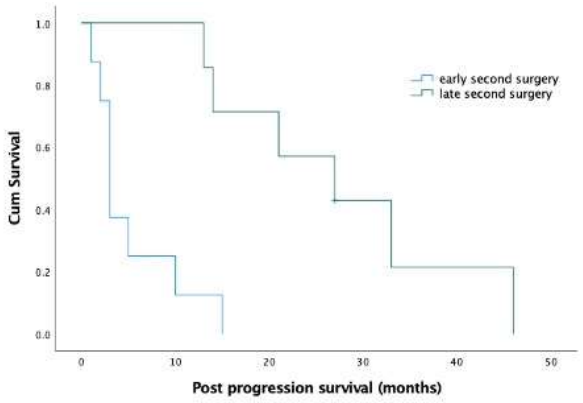
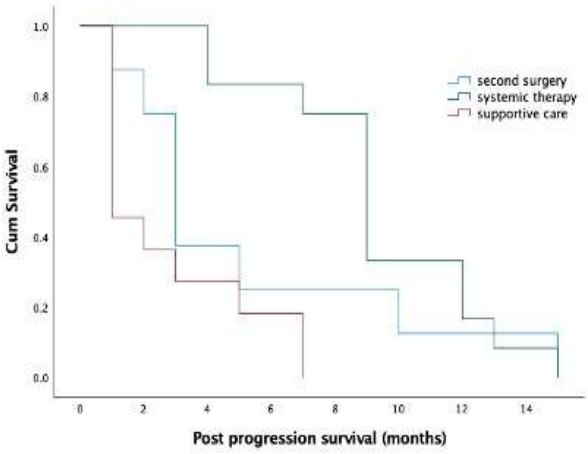


Figure 2: Kaplan Meier curves of surgery in early vs late progression

In multivariable analysis with factors with a p value of <0.05, we found progression time 9 months or less (p<0.001) and the use of supportive care after progression (p<0.001) as negative prognostic factors. Risk of death after progression was increased by a coefficient of 2.8 (95% CI: 1.6-5.0) in individuals who progressed ≤9 months in comparison to those who progressed later than 9 months. Re-operation (HR:0.20, 95% CI: 0.10-0.44), TMZ treatment (HR:0.37, 95% CI: 0.20-0.69) and BVC treatment arms (HR:0.26, 95% CI: 0.12-0.55) were associated with a lower risk of death after progression in comparison to supportive care arm (Figure 1).

Patients who underwent re-operation with or without adjuvant treatment had the longest



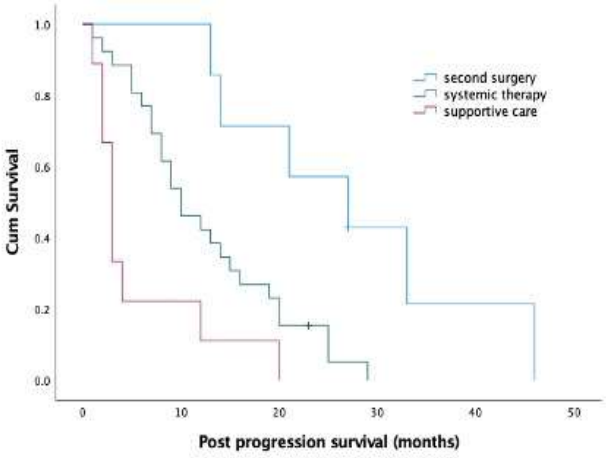


Figure 3: Kaplan Meier curves of treatments in a) early and b) late progression

PPS times (median 13 months, 95% CI: 1.6-24.4). However, when we consider the timing of re-operation; there was a significant PPS difference between early and late resections (median 3 months (95% CI:2.1-3.9) vs median 27 months (95% CI:11.6-42.4), p:0.001) (Figure 2).

In case of early progression; systemic therapy had a longer PPS than supportive care (median 9 vs 1 months, p<0.001). However there was no significant difference between re-operation and supportive care (median 3 vs 1 months, p=0.143) (Figure 3A).

In case of late progression; re-operation provided rates of PPS than systemic therapy (median 27 vs 10 months, p=0.005) and

supportive care (median 27 vs 3 months, p<0.001) (Figure 3B).

Discussion

Progression is inevitable after initial treatment of GBM. Today, however, there is no standard treatment for pGBM. In daily practice, if the clinical status of patient is suitable, the first option in mind is re-operation. However, re-operation is a highly controversial issue which has not been proven by randomized clinical trials. Analysis of 19 phase 2 studies by the North American Brain Tumor Consortium revealed no benefit of additional operations [9]. Nevertheless, the vast majority of literature supports the benefit of re-operation in pGBM [10-12]. A recent systemic review and a meta-analyse also advocates re-operation for progression [7,13]. Moreover, multiple resections have been found to increase survival. Chaichana et al. reported overall survival of 6.8, 15.5, 22.4 and 26.6 months after resections 1, 2, 3 and 4th, respectively [14]. In contrast, a study conducted in TMZ era revealed that patients with multiple resections were much younger and had higher perpormance status. And once adjusted for age, the benefit of multiple resections was no longer significant [15]. Recent data from the DIRECTOR cohort conclude that the benefit of re-operation is only with the removal of complete resection of the contrast-enhancing lesion [16]. Another study suggested that neutrophil/lymphocyte ratio (NLR) is a prognostic factor for PPS. The authors reported a median PPS of 9.7 months for NLR ≤ 4 and 5.9 months for NLR > 4 [17].

All these studies demonstrate the need for appropriate patient selection for re-treatments. Two studies analyzing data from phase 1 and 2 trials in North America and Europe reported that prognostic factors affected survival outcomes more than treatment modalities [18, 19]. Carson et al. identified age (≥50 vs. <50), 10 points increase in KPS and corticosteroid use in their recursive partitioning analysis for GBM. Gorlia et al. found World Health Organization (WHO) performance status, baseline steroids, tumor size (≤42mm vs. >42mm) and number of target lesions (1 vs. more than 1) as prognostic calculators. These prognostic factors are important to decide which treatment is avaliable for which patient. Several other studies also suggest that preoperative KPS scores are associated with higher OS time [20]. We have inadequate data of KPS at the time of first progression. However, an initial score higher than 70 to be correlated with better PPS trend after progression.

In this study, progression time greater than 9 months and re-treatment instead of supportive care were associated with longer survival after progression. Systemic therapies have similar PPS (9 and 10 months) when administered in case of early or late progression. However the benefit of re-operation reversed when it was incorporated into early progression. McGirt et al. emphasized the importance of gross total resection in both primary and secondary resections and found the benefit of a second surgery after 12 months of primary resection, but not earlier [21]. In a radiosurgery trial from Korea, radiosurgery and TMZ had a 15.5 month survival after progression. They also concluded that patients who progressed late, had better survival rates [22]. Conversely, Nava et al. and Ringel et al. found no prognostic effect of the progression time [23, 24]. The first found no benefit of reoperation in patient cohorts both before and after 2005. In addition, there was no 9-month threshold difference for resection results. However lower OS in the study (11.7 before TMZ and 12.9 after TMZ) may affect the outcomes. The latter found high survival rate (25 months), and good response to re-operation not dependent on the progression time. This may be due to the inclusion of well-selected patients with high KPS (median 90%) and low rapid progression rate (19% of patients had progression earlier than 5 months).

A recent study by Goldman et al pointed out the importance of time-dependent methodology for oncologic treatments [25]. They found that, re-operation was associated with a lower risk of death when timing was ignored (HR:0.62, 95% CI: 0.43-0.90, p=0.01). However, re-operation was associated with a higher risk of death after timing was taken into account (HR:2.19, 95% CI:1.47-3.28, p<0.001). This was also confirmed in a meta-analysis by Zhao et al. and a more recent single-instution study [7,26].

The evidence about the outcomes of surgical intervention are commonly from retrospective investigations. We need further researches to come to a conclusion and obtain higher-level evidence on the impact of surgery. The rate of re-operation in previous studies is 10-30%. More recent studies have reported higher rates of re-operation, possibly with improved surgical technics. However, care should be taken when making decisions in rapidly progressive cases. Second surgery can be considered in young patients with good performance status and progressive disease location that can be safely resectable in non-eloquent brain area. It helps relieving of symptoms quickly and may serve a better quality of life. In addition, information about the histopathological features of progressive disease may shed light on new therapeutic pathways.

Recently, improvements in radiotherapy setting allows a secondary radiation in the selected pGBM. A variety of re-irradiation studies have shown results comparable to other treatment modalities. Skeie et al. reported 12-month survival with radiosurgery [27]. Two studies from North America reported that 11-month survival with re-irradiation and no benefit of additional surgery before or after hypofractionated stereotactic radiation therapy [5, 28]. In our study population, only 2 patients were re-irradiated. This may be a result of primarily consideration of re-operation and systemic treatments and keeping radiation for residual tumors. However, the worsening of the patients' symptoms led to a decrease in the radiological detection rate of second relapses. Irradiation can be a good non-invasive option in both early and late progression.

Bevacizumab is a vascular endothelial growth factor inhibitor. We observed that BVC has similar efficiency with other treatment options. It may provide a better quality of life, particularly in early progression and when high doses of corticosteroid needed.

Our study has various limitations, one is that while chemotherapy efficacy has been shown to be dependent on the methylation of the promoter for MGMT, we did not have results for all of our patients and thus, we evaluated chemotherapy effectiveness without taking this factor into account. Similarly, IDH-1 status was unknown in half of the patients. Further studies will clarify the role of underlying molecular profiles in the pGBM treatment setting. Another limitation is the presence of combination therapies after progression, making it difficult to assess the efficacy of each. Finally, although some studies proposed 6-month progression free survival is a critical end point for evaluating the effectiveness of treatment [29], we were only able to prove a second relapse in 16 patients, and used PPS in this comparison.

Conclusion

In conclusion, Glioblastoma is a tumor with dismal survival even though efforts to increase treatment options and their effectiveness are being made. Standard treatments in progressive GBM are lacking. Survival after progression is considered to be shorter than a year and proper patient selection is crucial when deciding to proceed re-treatments. In particular, re-operation may not be a viable option for early progression, and should be discussed in multidisciplinary boards.

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Original Article

The Impact of the COVID-19 Pandemic on Head and Neck Cancer Practice –A Tertiary Health Care Center Experience

COVID-19 Pandemisi Sürecinin Baş Boyun Kanserleri Pratiğine Etkisi –Üçüncü Basamak Sağlık Merkezi Deneyimi

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ABSTRACT

Introduction: Coronavirus disease 2019 (COVID-19) has changed practice patterns of head and neck oncology services, as well as other service areas of health care. This study aims to describe the impact of COVID-19 on the services of the division of otorhinolaryngology, head and neck surgery at an academic tertiary referral hospital specialized in oncology. This is a single-center descriptive study conducted within the otorhinolaryngology department of a tertiary health care institution, which mainly provided service to oncological cases during the pandemic.

Methods: Data of cases over 18 years old on the numbers of outpatient visits, hospitalizations, otorhinolaryngological surgeries, and indications were obtained from March 1 to May 15 for 2019 and 2020 from hospital information management system. Data on preoperative test results of asymptomatic patients for COVID-19, going through for head and neck oncological surgeries were obtained from the same system.

Results: There is a decrease in the total number of outpatient visits in 2020 compared to 2019. (16814 vs. 7108, 57.7%). The numbers of hospitalizations and surgeries related to head and neck malignancies were increased despite the decrease in the total number of hospitalizations (278 vs. 129, $p < 0.001$) and in the total number of surgeries (231 vs. 111, $p < 0.001$). One of the 88 preoperative COVID-19 tests of asymptomatic patients was positive. No member of the staff got infected.

Discussion and Conclusion: Although there is a decrease in the number of patients in the 2020 period, the increase in the qualitative characteristics of the head and neck oncological procedures performed causes an increase in the difficulties / risks that health professionals face even though they do not work for pandemic services.

Keywords: Pandemics, Otolaryngology, COVID-19, Surgical Oncology, Health Care

ÖZET

Giriş ve Amaç: Koronavirüs hastalığı-2019 (COVID-19), sağlık hizmetinin diğer alanlarında olduğu gibi, baş ve boyun onkolojisi hizmetlerinin uygulama düzenlerini değiştirmiştir. Bu çalışma, COVID-19'un, onkolojide özelleşmiş üçüncü basamak bir akademik sağlık kurumunun Kulak Burun Boğaz, Baş ve Boyun Cerrahisi Bölümü hizmetleri üzerindeki etkisini açıklamayı amaçlamaktadır. Çalışmamız, pandemi sürecinde onkolojik olgulara hizmet veren üçüncü basamak bir sağlık kurumunun Kulak Burun Boğaz bölümünde yürütülen tek merkezli tanımlayıcı bir çalışmadır.

Yöntem ve Gereçler: Hastane bilgi yönetim sisteminden 2019 ve 2020 yıllarının 1 Mart-15 Mayıs tarihleri arasında 18 yaşından büyük olguların poliklinik başvurusu, hastaneye yatış ve kulak burun boğaz ameliyatları sayıları ile yatış ve ameliyat endikasyonlarına ilişkin veriler elde edildi. Baş ve boyun bölgesi onkolojik cerrahisi planlanan asemptomatik preoperatif hastaların COVID-19 test sonuçlarına ilişkin veriler aynı sistemden elde edildi.

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Bulgular: 2020'de poliklinik başvurularının toplam sayısında 2019'a göre azalma saptandı. (16814'e 7108, %57.7). Toplam hastaneye yatış sayısı (278'e 129, p <0.001) ve toplam ameliyat sayısındaki (231'e 111, p <0.001) azalmaya rağmen, baş ve boyun kanserleri nedeniyle olan ameliyat ve hastaneye yatış sayıları arttı. Asemptomatik hastaların ameliyat öncesi 88 COVID-19 testinden biri pozitifti. Bölüm personelinden enfekte olan kişi olmadı.
Tartışma ve Sonuç: 2020 döneminde hasta sayısında azalma olmakla birlikte baş ve boyun onkolojisine yönelik olarak uygulanan işlemlerin niteliksel özelliklerinde artış olması, sağlık profesyonellerinin pandemi hizmetleri için çalışmadığı durumlarda dahi karşı karşıya olduğu zorluk / risklerde artışa neden olmaktadır.

Anahtar kelimeler: Pandemi, otolaringoloji, COVID-19, Cerrahi Onkoloji, Sağlık hizmeti

Introduction

Coronavirus Disease – 2019 (COVID – 19) is a global problem and named as a pandemic by the World Health Organization on March 11, 2020. Since the very first cases of the disease in China, it is compared to the SARS (Severe Acute Respiratory Syndrome) and the MERS (Middle East Respiratory Syndrome) pandemics but it was clearly seen that the world is facing to a much bigger problem affecting all medical, social and economic issues.

Pandemics are periods in which the emergency and compulsory services that constitute the basics of health services should be prioritized. After the first COVID – 19 cases were reported, it was recommended that all hospitals review their pandemics plans, and to stratify urgent, non-delayable, and elective cases for each branch [1]. Head and neck cancer related procedures are prioritized by otolaryngology departments across the world. Clinical experiences provided in pandemics periods are also very valuable in terms of regulation of health services. Otorhinolaryngology units, providing either outpatient or inpatient services, were the first line to be affected by this pandemic because of the transmission way of the disease[2]. In the multimedia era, which is a main component of globalization, both patients and healthcare workers were getting information more quickly than the spread of the disease and this situation changed some practice patterns [3].

Herein, we discuss the early effects of the COVID-19 pandemic on our otorhino-laryngology service practice. Our institution is specialized in oncology long before the pandemic. The primary objectives were to examine the quantitative changes in outpatient visits, hospitalizations, and performed surgeries over the first months of the virus's impact within Turkey, in comparison with the prior year, paying extra attention to head and neck cancers. Secondarily, we analyzed trends of mentioned parameters in weekly changes.

Materials and methods

Setting

This was a retrospective, single-institution study conducted within the entirety of the department of otorhinolaryngology at a tertiary health care centerspecialized in oncology. The institution is an academic center that also includes community clinics. Cases requiring major surgeries are widely referred from all over the country, but also general otorhinolaryngological services provided to a city which has a population of near 6 million people. This study was approved by the Local Committee on Ethics (No. of meeting: 2021-01/937, January 13, 2021).

Period and Data

The first COVID-19 case was reported on March 11, 2020, in the country but a change of practice patterns, and postpone of elective surgeries on patient demand was seen before

that. Elective surgeries were temporarily halted by March 17, 2020, after the formal letter from the Ministry of Health.

As the focus of the study was on COVID-19's impact on otorhinolaryngology services and especially head and neck cancers, we queried data of from March 1 to May 15, 2020 (the 2020 period). For a comparison group, we queried the corresponding period from the previous year—March 1 to May 15, 2019 (the 2019 period). Dates were grouped into weeks 1–11 for each period from March 1, every seven days consisting of a week.

All patients attending the department aged over 18 years old (all database population) was included to minimize potential sources of bias. With the approval of the Local Committee on Ethics, writers had access to the database of the hospital information management system (HIMS) for data of all patients consisting date, diagnosis, performed procedures, results of preoperative tests.

Number of all outpatient visits within the otorhinolaryngology division were obtained from the HIMS. Detail of indications (hospitalizations and surgeries) were obtained fromHIMS database search with ICD-10 (International Classification of Disease) codes. Detailed data of performed surgeries were obtained by hospital information management system database search with Health Application Notification (HAN) codes. Data of surgeries were cross checked by departments operation registry book.

Hospitalizations were distributed to five major indication groups. The first group consists of patients with planned elective surgeries. The second group was hospitalizations before planned surgeries for head and neck cancers. The third group consists of patients who need hospitalization for advanced diagnostic procedures or biopsy (direct laryngoscopic examination, lymph node excision, imaging techniques requiring inpatient evaluation before a procedure, etc.). The fourth group of patients was hospitalized for debridement or drainage of infections like a peritonsillar abscess, chronic osteomyelitis of the jaw after radiotheraphy, etc. The fifth group of patients had otorhinolaryngological emergency conditions requiring medical or surgical treatment, like desaturation because of upper respiratory tract obstruction (common presenting symptom of head and neck cancers), post-tonsillectomy bleeding, or sudden sensorineural hearing loss.

Surgical procedures were distributed to four groups. The first three groups are the same as hospitalization groups. The fourth and fifth groups were combined for statistical purposes: surgeries for debridement and emergencies consist the fourth group.

Service providing specialists were general otorhinolaryngologists as subspecialties are not yet established in the country. All have at least 5 years of experience in head and neck surgery.

Statistical Analysis

Chi-square ($\chi 2$) test was used to compare categorical data. Statistical analysis was performed via IBM SPSS for Windows version 26.0 and significance was set at $p<0.05$.

Results

Outpatient services

Between March 1 and May 15, 2019; 16814 visits are completed within the otorhinolaryngology division. This was distributed to 14 providers: seven otolaryngologists (one professor, two associate professors, four specialists), four residents (all general practitioner, medical doctors), one speech and language pathologist, and two audiologists. In the COVID – 19 period (March 1 – May 15, 2020) 7108 outpatient visits were completed by the same providers. There is more than half

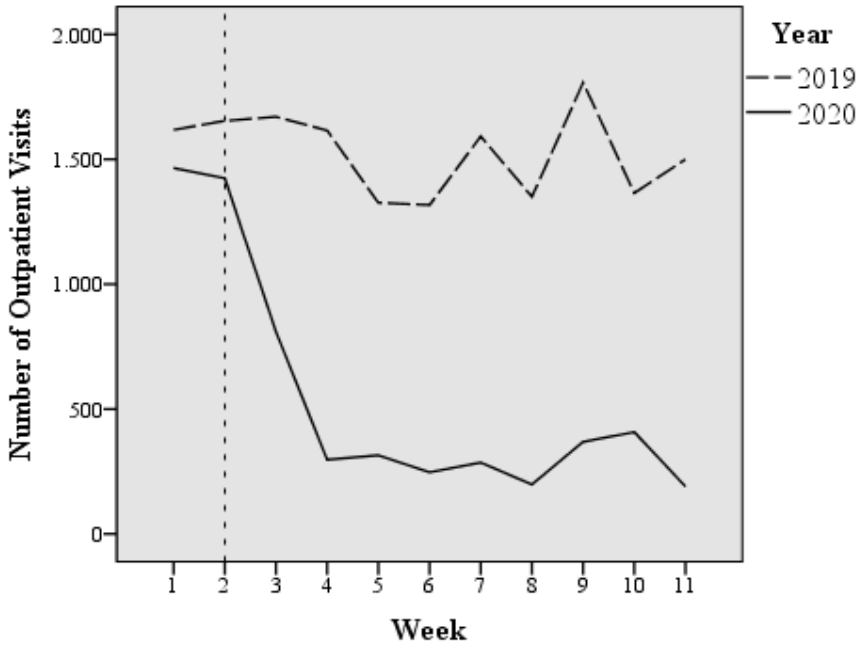

Figure 1. The weekly breakdown of completed outpatient visits in the 2019 and 2020 periods. Note that the first case was seen on March 11, 2020 (week 2, vertical dotted line).

decrease (57.7%) of closed outpatient visits (Figure 1).

Hospitalizations

Between March 1 and May 15, 278 patients were hospitalized in 2019. Between March 1 and May 15, 129 patients were hospitalized in 2020. On chi-square analysis, a smaller number of patients were hospitalized in 2020 period as compared with 2019 period. (129 vs 278, $p < 0.001$). The decrease in the number of hospitalized patients is obviously seen on weekly basis (Figure 2). Distribution of hospitalization indications has also changed by the pandemic, mainly because of elective cases (Figure 3).

Surgical procedures and preoperative tests

Between March 1 and May 15, 231 otorhino-laryngological surgeries were performed in the operating room in 2019. Between March 1 and May 15, 111 otorhinolaryngological surgeries were performed in the operating room 2020. On chi-square analysis, there is a significant decrease in the number of operations in 2020 as compared with the number of operations is in 2019 (111 vs 231, $p<0.001$). This decrease can be seen in the weekly breakdown of surgeries (Figure 4).

Surgical indications were also changed. In 2019 most of the surgeries were elective (71.9%), whereas surgeries for malignancies take the first place (40.5%) in 2020 (Figure 5).

The most common surgical procedure in the 2020 period was a major head and neck cancer surgery:14 neck dissections of eight patients (five total laryngectomy + bilateral neck dissection, two hemiglossectomy + unilateral neck dissection, one lower lip midline carcinoma excision + bilateral anterolateral neck dissection) were performed. Also, one patient had neck dissection within the COMMANDO Procedure (COMbined MAndibulectomy and Neck Dissection Operation) and pectoralis major muscle skin flap repair. Another patient with oral cavity (gingiva-mandible) carcinoma diagnosed just

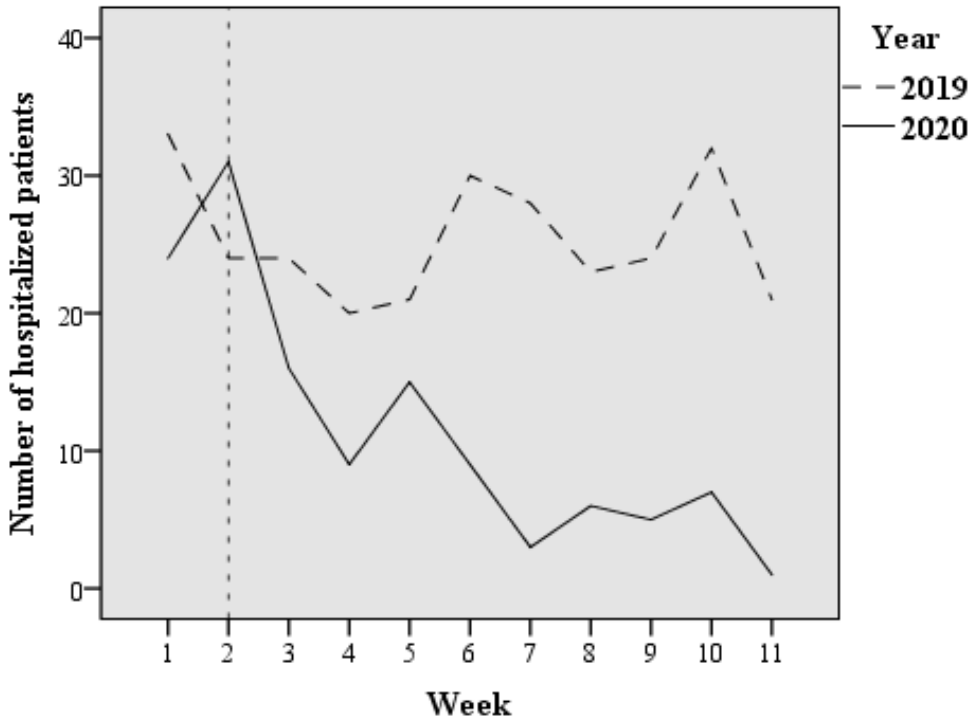

Figure 2. The weekly breakdown of hospitalizations in the 2019 and 2020 periods. Note that the first case was seen on March 11, 2020 (week 2, vertical dotted line).

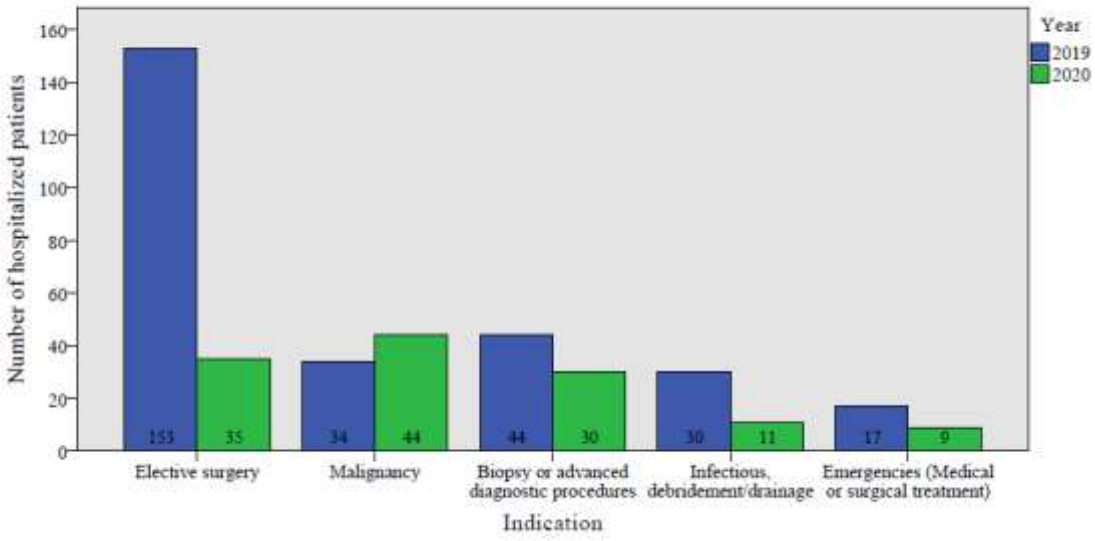

Figure 3. Distribution of hospitalization indications in the 2019 and 2020 periods.

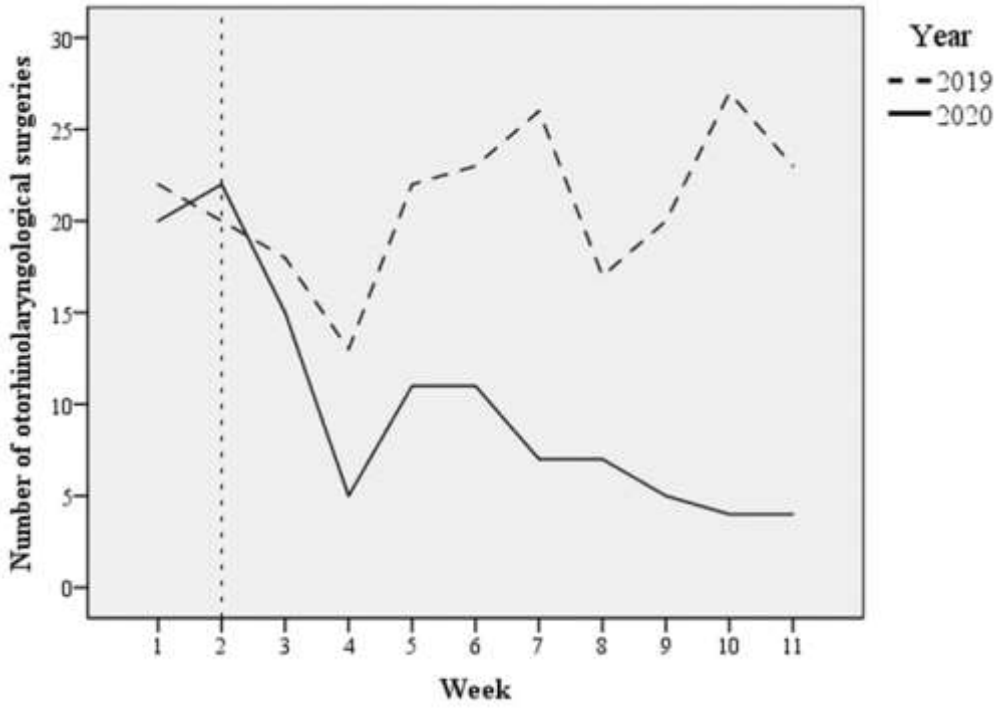

Figure 4. The weekly breakdown of otorhinolaryngological surgeries performed in the operating room in 2019 and 2020. Note that the first case was seen on March 11, 2020 (week 2, vertical dotted line).

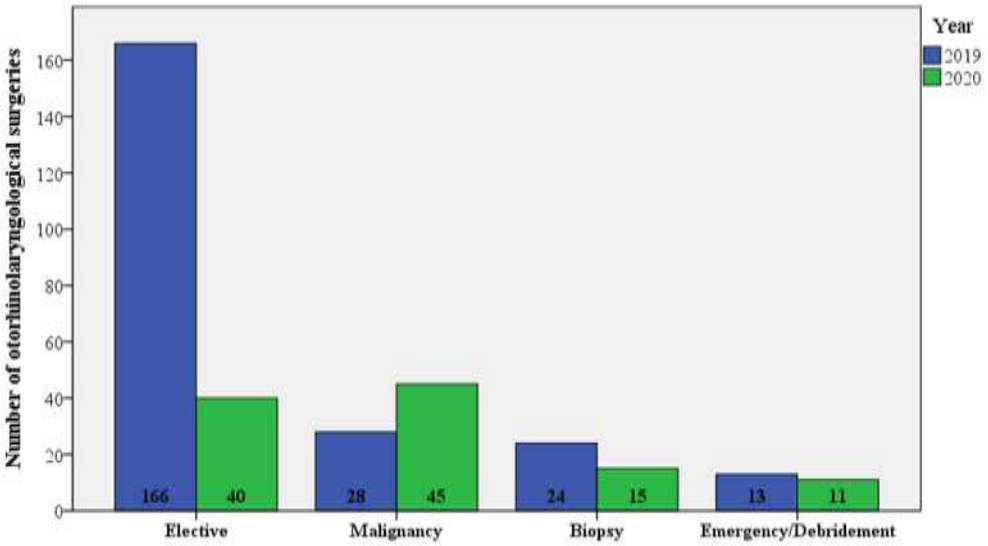

Figure 5. Distribution of indications for surgery in the 2019 and 2020 periods. 40 elective surgeries were performed between March 1 and March 17, 2020

before the outbreak, 10 years after tongue carcinoma treatment on the same side, had lateral segmental mandibulectomy for tumor resection and pectoralis major muscle skin flap repair.

The second most common surgical procedure in the 2020 period was direct laryngoscopy and laryngeal biopsy of 11 patients with suspected malignancy. Two of them had a tracheotomy in the same session. Besides, one

patient with respiratory distress and one patient with prolonged intubation received a tracheotomy.

Total laryngectomy for five patients, partial / hemiglossectomy due to tongue carcinoma (+/-neck dissection) for four patients was performed in the 2020 period. Other surgeries include resection and repair of facial skin cancers, debridement of invasive fungal rhinosinusitis in the same period.

To protect staff and to ensure continuity of healthcare, preoperative polymerase chain reaction (PCR) testing for COVID-19 was provided for asymptomatic head and neck cancer patients by the ninth week of the 2020 period, after discussion with the institutional COVID-19 committee. Samples for PCR tests were collected by nasopharyngeal/ oropharyngeal combined swabs. A total number of 12 PCR tests were performed, 1 resulted positive. The staff members involved with the patient were isolated at home with symptom monitoring, all had PCR tests done on the fifth day, all results were negative. After the start of the "normalization period" (June 1, 2020), to July 29, 2020, an additional number of ,76 PCR tests were performed and all resulted negative. The latter 76 test were performed in consistency with the recommendation of the Ministry of Health, for patients with planned undelayable major surgeries.

Discussion

The COVID – 19 pandemics has changed the structure of health care globally and continues to do so with every piece of knowledge obtained about it [4]. Using different platforms, physicians are trying to get information about each other's practices to give the best decisions about the model of service, sometimes requiring new methods for sharing updated knowledge [5]. The knowledge about the virus, ways of treatment, and protection is updating and characteristics involving otorhinolaryngology are widely discussed[6] but we have to put this information into practice.

An outbreak period caused by an aerosol-borne disease, people with flu-like symptoms are stratified as "high-risk patients"[7]. This group of patients constitutes an important part of otorhinolaryngology outpatient visits. Another important problem is that COVID-19 cases can be asymptomatic at a rate of 5-80%[8]. Therefore, high risk is not only limited to pandemic outpatient services but also directly related to the nature of the examinations or procedures performed.In all units of the department performing outpatient otolaryngological services, personal protective equipment (PPE) consist of N95/FFP2 mask plus surgical mask, goggles/ visor, bonnet, double gloves and disposable gowns recommended by local association of otolaryngology head and neck surgery [9] are routinely used for procedures described as "close contact" by the "COVID-19 Advisory Committee of Ministry of Health of Turkey".

During the 2020 period, the number of outpatient visits is decreased as expected and observed in pandemics [4,10,11]. This may be both from the reduction of appointments and hesitation of patients to go to a hospital building during the outbreak. Despite the dramatic decrease compared to the year before, an average of 120 outpatient visits per day are completed, mainly for head and neck cancers. Although for the first days we had some organizational issues, we did not face any shortage of appropriate PPE recommended by national or international guidelines [9,12]. If the PPE mentioned above fits the healthcare worker properly, hypercapnia and associated symptoms develop after a while. This situation during the outpatient service causes additional strain, breaks between visits should be maintained. Transparent booths for patient examination were used when appropriate. Other valuable precautions to ensure the protection of staff is

flexible working order which also prevented cross-contamination and screening of all patients who were asked to wear surgical masks before the visit. All patients were screened for COVID-19 by measuring body temperatures without contact and filling the inquiry form prepared by the Ministry of Health, even if they are asymptomatic. If any patient has susceptible signs or symptoms, referral to the pandemic outpatient clinic is provided. As no member of the staff got infected during the period, these precautions seem to be protective for the outpatient clinic staff.

Most hospitalizations were for surgical purposes both in the 2019 and 2020 periods. The most prominent cause in the dramatic decreases in the number of hospitalized patients and in the number of otorhino-laryngological surgeries was the reduction of elective indications. These are common findings for pandemics [10,13]. By the fourth week of the 2020 period, there is a slight increase in the number of hospitalizations and surgeries. With a decision of the provincial health directorate, COVID-19 positive patients were cohorted to other specified hospitals in the city, and cases from those hospitals referred to our institution for major otorhinolaryngological surgeries, most of which are head and neck cancer. So, as a difference from other institutions' experiences [14], there was an increase in the number of surgeries for malignancies in the 2020 period. This caused extra effort of the staff with mentioned PPE but in the end, patients received appropriate treatment on time with no extra complications.

Our oncological practice also changed by the pandemic. As COVID -19 causes increased mortality in post-operative period[15], some head and neck carcinoma cases were referred to Radiotherapy Department directly instead of performing surgery, if the expected treatment success rate is close for each option. Surgical treatment of squamous cell cancers of head and neck is recommended to continue in the pandemic period if postponing causes progression in the stage of the disease or results more aggressive approach [16]. Head and neck cancer operations involve upper aerodigestive tract surgery, causing extra aerosol production which increases the risk of viral exposure. A significant proportion of patients may be asymptomatic so temperature measurements and symptom inquiries may not work for these cases. After transnasal surgery of an asymptomatic case from China, over 14 health care workers have reported being infected [17]. Since the normalization period has started on June 1, 2020, even asymptomatic cases are having PCR tests before major surgeries in our institution, after the recommendations of the national advisory board. Appropriate filiation of the case with the isolation of the health care workers involved to the case may save money and health by performing preoperative PCR testing of asymptomatic patients. But in our experience (from March 1, 2020, to July 29, 2020), a total number of 88 preoperative PCR tests for asymptomatic patients were performed, one resulted positive. No member of the otolaryngology operating room staff developed symptoms or had positive PCR test results up to July 29, 2020. More researches should be done on the concept of preoperative PCR testing, as we are performing surgeries with the mentioned PPE even the PCR test is negative.

As mentioned before appropriate use of PPE by the entire otorhinolaryngological service providing team is very important, even with negative PCR results. We did not use powered air-purifying respirator (PAPR) or disposable overalls for patients with undetermined COVID-19 status. Performing major oto-rhinolaryngological surgeries with overalls + PAPR for hours may be an extra physical burden to the surgeon besides the costs.

Research should be conducted on this issue.

Because of the feature of the hospital mentioned above, we have no findings to present on proven COVID-19 positive cases. Another limitation of the study is the lack of telemedicine data which is still a matter of debate for some otorhinolaryngological services.

Conclusion

In the current study, the COVID-19 outbreak experience of a tertiary otorhinolaryngology head and neck surgery department is explained. In general, the number of patients by terms of outpatient visits, hospitalizations, and performed surgeries are reduced but the qualitative features of the procedures performed are increased, mainly as a result of the increase in head and neck malignancy related procedures. Because of these changes in practice patterns, the difficulties/risks that otorhinolaryngology healthcare professionals are facing are increased in the 2020 period compared to the 2019 period.

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Original Article

Accuracy of Preoperative Computed Tomography for Lymph Node Status Screening in Colon Cancer

Kolon Kanseri Hastalarında Lenf Nodu Durumunu Görüntülemede Preoperatif Bilgisayarlı Tomografinin Doğruluğu

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ABSTRACT

Introduction: Our aim is to determine the value of a pre-operative Computed Tomography (CT) scan for the assessment of lymph node status in patients diagnosed with colon cancer by comparing between radiological N-stage and histopathological N-stage.

Methods: After approving by local ethics committee, an experinced radiologist reviewed all pre-operative CT scans of patients diagnosed with colon cancer retrospectively, between January 2014 and December 2018. The CT scans were examined for any signs of regional lymphatic spread which was defined as lymph nodes exceeding 1 cm, clusters of ≥3 lymph nodes or a combination of the two. The results were compared with the histopathological N-stage. The diffrences in comparison were eveluated statistically and positive predictive value (PPV), negative predictive value (NPV), sensitivity, specificity and accuracy were calculated.

Results: We included 184 patients in our study. The statistical values of PPV, NPV, sensitivity, specificity, and accuracy of detecting regional lymph nodes metastases were 65.6%, 75%, 58.3%, 80.3% and 71.7%, respectively. The assessment of lymph node status with CT scans resulted in a moderate sensitivity, specificity and accuracy for both subgroups, defined as emergency and tumor localization subgroups.

Discussion and Conclusion: Although our study group is relatively large and homogeneous compared to previous studies, the obtained results in the evaluation of patients with colon cancer with preoperative CT does not seem to be satisfactory. Before making the treatment decisions according to the appearance of lymph nodes in colon cancer patients on CT images, the diagnostic accuracy needs strong improvement, such as thinner axial slices and three-dimensional reconstruction methods.

Keywords: Colon cancer, Lymph nodes status, Computed tomography, Preoperative staging

ÖZET

Giriş ve Amaç: Çalışmamızda kolon kanseri tanılı hastalarda radyolojik ve histopatolojik lenf nodu evresini kıyaslayarak preoperatif bilgisayarlı tomografi (BT) ile taramanın lenf nodu durumunu belirlemedeki değerini belirlemeyi amaçladık.

Yöntem ve Gereçler: Ocak 2014 ve Aralık 2018 tarihleri arasında kolon kanseri tanısı almış olan hastaların preoperatif BT taramaları alanında deneyimli bir radyolog tarafından geriye dönük olarak

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incelendi. Görüntülerde bölgesel lenf nodu yayılımının göstergesi olarak 1cm'den büyük lenf nodu, ≥3 kümelenmiş lenf nodları veya her ikisinin de mevcut olması kabul edilmiştir. Sonuçlar histopatolojik lenf nodu evresi ile kıyaslandı. Ardından, pozitif prediktif değer (PPD), negatif prediktif değer (NPD), sensitivite, spesifisite ve doğruluk değerleri hesaplandı.

Bulgular: Çalışmamıza 184 hasta dahil olmuştur. Bölgesel lenf nodu metastazının tespitinde tespit edilen PPD, NPD, sensitivite, spesifisite ve doğruluk değerleri sırasıyla %65.6, %75, %58.3, %80.3 ve %71.7 idi. Acil ve tümör yerleşimi gibi alt gruplarda BT ile lenf nodunun değerlendirilmesinde ise BT'nin orta düzey sensitivite, spesifisite ve doğruluğa sahip olduğu görüldü.

Tartışma ve Sonuç: Çalışmamız daha önceden yapılmış olan çalışmalara göre nispeten daha çok hasta içermesi ve daha homojen yapıda olmasına rağmen, kolon kanseri hastalarının preoperatif BT ile lenf nodu durumunu değerlendirilmesinde tatmin edici sonuçlara ulaşılamamıştır. BT görüntülemedeki lenf nodunu görünümüne göre tedaviye yön vermede tanısal doğruluğu arttıracak ince aksiyal kesitler ve üç-boyutlu rekonstruksiyon yöntemleri gibi güçlü gelişmelere gereksinim vardır.

Anahtar Kelimeler: Kolon kanseri, Lenf nodu durumu, Bilgisayarlı tomografi, Preoperatif evreleme

Introduction

Colorectal Cancer (CRC) is the most common diagnosed gastrointestinal neoplasm in western world. Approximately 70% of cases are located to colon and the treatment for resectable CRC is curative surgery with adequate lymph node (LN) dissection.

Preoperative chemoradiotherapy is the approved treatment to make smaller the tumor size and prevent local recurrences for high-risk rectum cancer [1]. American Joint Committee on Cancer (AJCC) recommends adjuvant chemotherapy for stage 3 colon cancer [2]. Colorectal surgeons have began to prefer neoadjuvant chemotherapy (NAC) for high-risk colon cancer preoperatively to prevent local recurrences after surgery; however, determining the appropriate patient for neoadjuvant therapy became more important [3]. Endorectal ultrasonography (EUS) and magnetic resonance imaging (MRI) are used to stage rectum cancer patients for NAC, but EUS is not appropriate for colon neoplasms, and also positron emission tomography (PET) and MRI have a low sensitivity [4,5]. Therefore, CT seems to be the only imaging modality to determine the distant metastasis and LN status and to select appropriately colon cancer patients for NAC. We aimed to reveal the effect of preoperative CT in diagnosing the LN status in colon cancer.

Material and Methods

After the local ethics committee approval (25.12.2017-44/15), the hospital records of elective and emergency curative surgery performed patients between January 2014 and December 2018 were analyzed retropectively. Rectum neoplasms, NAC performed patients, synchronous neoplasms, stage 4 neoplasms, palliative surgery (bypass or enterostomy) performed patients, recurrent colon neoplasms, less than 12 LN dissected patients and CT images missing patients were excluded from the study. CT images were examined by a single radiologist (YA) with more than 10 years of abdominal CT experience by knowing only the primary tumor localization of the patient. Radiological examination defined positive LN as; diameter >1 cm regional LN and\or count of ≥3 clustered regional LN. Intravenous (IV) and oral contrast agents were performed for all elective cases, and only IV contrast agent was performed for emergency cases.

The abdomen of the patients was scanned from diaphragm to pelvis with Toshiba Alexion 16 slice CT Scanner. Iohexol 300 mg I/mL was administered intravenously according to the weight of the patient with an

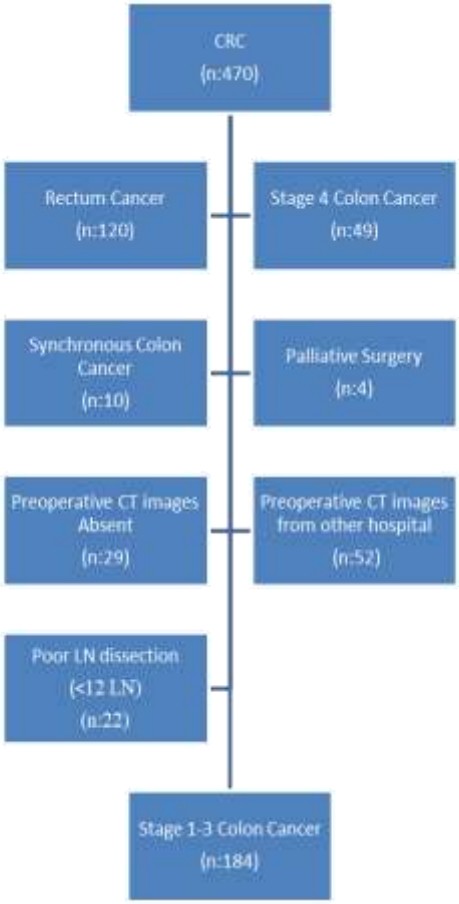

Figure 1. Inclusion and exclusion criteria

Imaxeon syringe. All CT scans were viewed on 3 mm axial sliced images. The maximum short axis in the axial plane was measured. All captured images were recorded in the hospital's PACS system.

Histopathological examinations of resected colon specimens were performed compatible with standard references, and LN were isolated by the only dissection, without oil cleaning techniques. The differentiation of pN1 and pN2 was made compatible with TNM classification, 8th edition, in subgroup analysis [6].

The data analyses were done with IBM SPSS Statistics 17.0 (IBM Corporation, Armonk, NY, USA) package software. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of CT were analyzed.

Table 1. The patients and neoplasm demographics

		N (%)
Stage 1-3 colon cancer patients		184
Gender		
	Male	110(59.7)
	Female	74(40.3)
Age		
	Median	66.0
	Range	38-91
Surgery		
	Emergency	53(28.8)
	Elective	131(71.2)
Localization		
	Right colon	56(30.3)
	Transvers colon	9(4.9)
	Left colon	31(16.8)
	Sigmoid colon	44(23.8)
	Rectosigmoid	44(23.8)
Tumor size		
	Median	4.7
	Range	2-13
T		
	T1	10 (5.4)
	T2	19 (10.3)
	T3	111 (60.3)
	T4	44 (23.9)
N		
	N0	112 (60.8)
	N1	55(29.8)
	N2	17 (9.2)
TNM		
	Stage 1	24 (13.0)
	Stage 2	87 (47.3)
	Stage 3	73 (39.7)

Results

A total of 470 diagnosed CRC patients were operated between January 2014 and December 2018. After exclusions, 184 TNM stage 1-3 colon cancer patients who had curative surgery, were included in the study (Figure 1).

The patients and neoplasm demographics were listed in Table 1. T3 + T4 tumours (155\184) were 84.2 % of the population and 39.1 % (N1+N2; 72/184) of cases had LN involvement (Table 1).

Table 2. Distribution of patients according to histopathological and radiological findings

		histopathological lymph node involvement		
		pLN+	pLN-	
preoperative CT images	rLN+	True positive(n): 42	False positive(n):22	PPV*: 65.6%
	rLN-	False negative(n):30	True negative(n):90	NPV**:75.0%
		Sensitivity: 58.3%	Spesificity: 80.3%	

pLN+: pathological lymph node+; pLN-: pathological lymph node-; rLN+: radiological lymph node+; rLN-: radiological lymph node-*positive predictive value,** negative predictive value

Table 3. PPV, NPV, Sensitivity, Specificity, and Accuracy

	PPV*	NPV**	Sensitivity	Specificity	Accuracy
Total (n:184)	65.6%	75.0%	58.3%	80.3%	71.7%
T1+T2 (n:29)	25.0%	84.0%	20.0%	87.5%	75.8%
T3+T4 (n: 155)	68.3%	72.6%	61.1%	78.4%	70.9%
Elective Surgery (n:131)	67.3%	70.7%	57.8%	78.3%	69.4%
Emergency Surgery (n:53)	60.0%	84.2%	60.0%	84.2%	77.3%
Right+Transvers Colon (n:65)	63.3%	80.0%	73.0%	71.7%	72.3%
Left+Sigmoid+Rectosigmoid Colon (n:119)	67.6%	72.9%	50.0%	84.9%	71.4%

*positive predictive value,** negative predictive value

A comparison of preoperative CT images with histopathological findings revealed true positive and negative, false positive and negative cases (Table 2). We found 42 true positives, 90 true negatives, 22 false positives, and 30 false negatives.

Diagnosis of malign LN's PPV, NPV, sensitivity, specificity and accuracy rates were; 65.6% (95% CI 55.5%- 74.4%), 75% (95% CI 69.22%-80%), 58.3% (95% CI 46.11%-69.8%), 80.3% (95% CI 71.7%-87.2%) and 71.7% (95% CI 64.6%-78.1%), respectively (Table 3).

Discussion

To date, there has been a tendency on multimodal therapies, including chemotherapy, radiotherapy, and surgical procedures to surgery alone therapies for different colon tumor stages [7]. One of the conspicous alteration of these treatment modalities is the NAC for local advanced and LN involved colon neoplasms, which gains significantly increased survival rates [8]. NAC has become the gold standard treatment modality for the esophagus, stomach, rectum, and breast cancer; however, it has been arguable validity for colon cancer.

CT is widely used to evaluate the primary tumoral lesions and distant metastasis for preoperative staging, in addition LN metastasis could be diagnosed accurately by CT preoperatively. The previous studies stated that; the sensitivity of CT for T3 and T4 tumors was above 90% in the evaluation of pathological T [9], while the sensitivity for

detection of malignant LN ranged between 13% to 92% [10-12].

CT axial sliced images vary from 5mm-8mm to 10mm in different studies; thus, limitations and discussions in evaluating LN with CT are associated with these variable and wide-ranged results. Dighe et al. stated that results were better in determining the metastatic LN when the axial sliced was ≤5 mm with their metanalysis study [13].

The definition of metastatic LN's image on CT is variable in different studies. LN's diameter size >5 mm [14], size >8mm [15], size >1cm [16], ≥3 number of LN [16], and large LN with irregular contours [14] were defined as metastatic LN in some studies. However, inflammatory large-sized LN, metastatic small-sized, and non-clustering LN have revealed that it is misleading to use dimensional or morphological findings alone in the evaluation of metastatic LN [17].

Therefore, researchers have began to conduct studies using more than one findings in the evaluation of metastatic LN. Rolven et al. reported that the CT sensitivity was 85%, and specificity was 75% in detecting stage 3 colon cancer, using together with the internal heterogeneity and irregular LN border [18]. In another study where LN size >5 mm and\or irregular contours defined as metastatic LN, CT sensitivity and specificity were 64% and 53%, respectively [19].

Measuring the axial length of LN is another commonly used method for LN evaluation; however, an LN shorter than 1 cm in axial sequence may be longer than 1 cm in sagittal or coronal sequences. Kanamoto et al. suggested two or three-dimensional reconstruction methods to increase sensitivity, specificity, and accuracy rates in their studies [20], but these methods seems to be time-consuming. They also stated that in axial sequence, CT images using short\long axis ratio ≥0.8 to define metastatic LN raised sensitivity to 87% and specificity to 80% [20].

The low rate of intraabdominal adipose tissue complicates to distinguish the infiltrated tumor into the pericolonic adipose tissue from local LN metastasis, and it is accepted that evaluation of LN status with CT will be difficult for these patients. Acute obstructive colon neoplasms with over dilated colon segments will distort the CT images, leading LN evaluation. On the other hand, Sjovall et al. reported that there was no difference between CT evaluation of primary colon tumor and histopathological T and N depending on criterias such as age, gender, BMI, emergency surgery, and localization of the tumor [21]. We used only intravenous contrast for emergency surgery and both intravenous and oral contrast for elective surgery patients during CT. We also observed that there was no significant proportional difference in terms of lymph node evaluation between patients who underwent emergency and elective surgery. Likewise, there was no abnormal change according to the tumor location according to current study. According to our radiologic criteria, the assessment of LN status with CT resulted in a moderate sensitivity and specificity for both elective and emergent patients.

Preoperative CT has not been able to get desired results in the evaluation of LN. Once we defined the metastatic LN as diameter >1 cm and\or ≥3 clustered LN, 11.9% (22/184) patients would get unnecessary NAC and 16.3% (30/184) patients who were a candidate for NAC would not get NAC in our study. The T1+T2 subgroup analysis revealed that; PPV and sensitivity were significantly lower than the other groups. These results indicated that; it is necessary to determine the structural findings as well as the dimensional findings, even T status, and all findings together in determining the metastatic LN. Besides,

radiologic criteria for lymph node metastases on CT in colon cancer resulted in moderate specificity and sensitivity both in left sided and right sided disease.

The retrospective nature of the study, single radiologist examination, and the limited number of patients are the limitation of our study.

In conclusion, although CT has been performed via thinner axial sliced images and relatively more homogeneous groups compared to literature, the results we obtained in the evaluation of patients with colon cancer with preoperative CT are not satisfactory. In order to reach an improvement in universal definition and accuracy for the different treatment options, there is a need for further studies with a prospective, more significant number of patient groups, using thin axial sliced and three-dimensional reconstruction methods and examining more than one findings for colon cancer patients' pre-operative LN examination with CT.

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Original Article

Predictive Role of Blood Flow Characteristics in the Detection of Malignant Breast Lesions: A Prospective Study

Malign Meme Lezyonlarının Saptanmasında Kan Akışı Özelliklerinin Prediktif Rolü: Prospektif Bir Çalışma

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ABSTRACT

Introduction: The aim of this study is to investigate the predictive feature of lateral thoracic artery (LTA) and internal thoracic artery (ITA) power doppler ultrasound examination results of patients with malignant breast lesions.

Material and Methods: This is a prospective case-control study in which 47 patients with suspicious lesions detected by ultrasonography and mammography and diagnosed with pathologically invasive breast carcinoma between 2018-2020 were included in a tertiary hospital. The breast with invasive carcinoma and the intact breast of the same patient were evaluated with LTA and ITA by power doppler ultrasonography. Healthy breast was the control group.

Results: In the diagnostic statistical tests; the optimum cut-off value for LTA peak systolic flow (PSF) value is 19.6 cm/s, sensitivity of this value is 81.3% and specificity is 95.8%. The optimal cut-off value for LTA resistive index (RI) value was 0.805, sensitivity of this value was 91.7% and specificity was 95.8%. For the detection of invasive breast carcinoma in the contralateral breast, the sensitivity of ITA peak diastolic flow (PDF) below 8.35 is 77.1% and the specificity is 79.2%.

Conclusion: To the best of our knowledge, this study is the first publication to examine the flow patterns of internal ITA and LTA together. This study presents new quantitative diagnostic tests that can be used to detect breast cancer, are easily accessible, applicable, and have high sensitivity and specificity.

Keywords: Breast Tumors; Blood Flow Velocity, Doppler Ultrasound Imaging, Thoracic Arteries

ÖZET

Giriş: Bu çalışmanın amacı malign meme lezyonu olan hastaların lateral torasik arter (LTA) ve internal torasik arter (İTA) power doppler ultrason inceleme sonuçlarının prediktif özelliğini araştırmaktır.

Gereç ve Yöntemler: Bu çalışma, 2018-2020 yılları arasında ultrasonografi ve mamografi ile şüpheli lezyonları saptanan ve patolojik invaziv meme kanseri tanısı konan 47 hastanın üçüncü basamak bir hastanede dahil edildiği prospektif bir vaka kontrol çalışmasıdır. Aynı hastanın invaziv karsinom tanısı alan memede ve sağlıklı memede power doppler ultrasonografi ile LTA ve İTA ile değerlendirildi. Sağlıklı meme kontrol grubuydu.

Bulgular: Tanısal istatistiksel testlerde; LTA PSF değeri için optimum cut-off değeri 19.6 cm/s, bu değerin duyarlılığı %81.3 ve özgüllüğü %95.8'dir. LTA RI değeri için optimal cut-off değeri 0.805, bu değerin duyarlılığı %91.7 ve özgüllüğü %95.8 idi. Karşı memede invaziv meme kanseri tespiti için ITA PDF'nın 8.35'in altındaki duyarlılığı %77.1, özgüllüğü %79.2'dir.

Sonuç: Bildiğimiz kadarıyla, bu çalışma dahili ITA ve LTA'nın akış modellerini birlikte inceleyen ilk yayındır. Bu çalışma, meme kanserini tespit etmek için kullanılabilecek, kolay erişilebilir, uygulanabilir, yüksek duyarlılık ve özgüllüğe sahip yeni kantitatif tanı testleri sunmaktadır.

Anahtar Kelimeler: Meme tümörleri, Kan Akış Hızı, Doppler Ultrason Görüntüleme, Torasik Arterler

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Introduction

The most common malignancy in women is breast cancer and is the second most common cause of death after lung cancer worldwide [1]. Early diagnosis is the most important factor that increases survival in breast cancer, and the prognosis of the disease is directly related to the stage at the time of diagnosis [2]. Screening with mammography at a rate of 16-40% plays a role in the reduction of breast cancer deaths among women aged 40-74 [3,4]. However, it has been reported that mammography rates decrease as the breast density increases [5]. Screening by mammography alone may be insufficient in these women [6]

For the evaluation of breast lesions, breast ultrasonography is an important imaging method that complements mammography and has high sensitivity and specificity. It is easily possible to evaluate cystic lesions in the breast and to evaluate peripherally located breast lesions with breast ultrasonography [7-9]. However, the quality of ultrasound images for ultrasonographic evaluation is affected by many factors such as contrast and signal to noise ratio, acoustic shadowing and enhancement artifacts. In addition, it is subjective because it depends on the experience and skill levels of the person performing ultrasonography (10). Quantitative ultrasonographic parameters are investigated in order to decrease misdiagnosis in breast ultrasonography and to eliminate this subjective evaluation [11].

Internal thoracic artery (ITA), lateral thoracic artery (LTA) and internal mammarian artery (IMA) play a role in breast feeding. ITA nourishes the breast and anterior chest wall. IMA feeds the breast through the posterior and anterior medial branches and LTA feeds the lateral part of the chest [12,13]. Power doppler ultrasound (PDUS), on the other hand, has a diagnostic value in distinguishing benign and malignant masses of lesions by evaluating the blood flow and vascularization of solid lesions in gray scale ultrasonography (US) findings [14,15]. However, there are not enough studies investigating values such as resistive index (RI), pulsatility index (PI) of ITA and LTA in patients with malignant breast lesions.

The aim of this study is to investigate the predictive feature of LTA and ITA PDUS examination results of patients with malignant breast lesions.

Materials and Methods

Ethic Approval and Patients

This is a prospective case-control study in which 47 patients with suspicious lesions detected by ultrasonography and mammography and diagnosed with pathologically invasive breast carcinoma between 2018-2020 were included in the Adıyaman Training and Research Hospital. Local ethics committee approval was obtained for the study. The study was carried out in accordance with the Declaration of Helsinki, and a signed consent form was obtained from all participants or their legal guardians.

Being 18 years of age or younger, women diagnosed with malignancy at the time of diagnosis or previously, having a history of breast surgery in the last 12 months, receiving radiotherapy and chemotherapy in the last 12 months, infectious diseases such as periductal mastitis and granulomatous mastitis that have been treated in the last 12 months, having illnesses and being in the lactation period were exclusion criteria from the study.

Ultrasonographic examination

The evaluation of the patients was performed on a GE Logiq S8 (GE Healthcare, Milwaukee, WI, USA) ultrasound device using a 12-MHz linear probe.

Patients were evaluated primarily by US examination and B-Mode US examination by two radiologists with 5 years and 15 years of

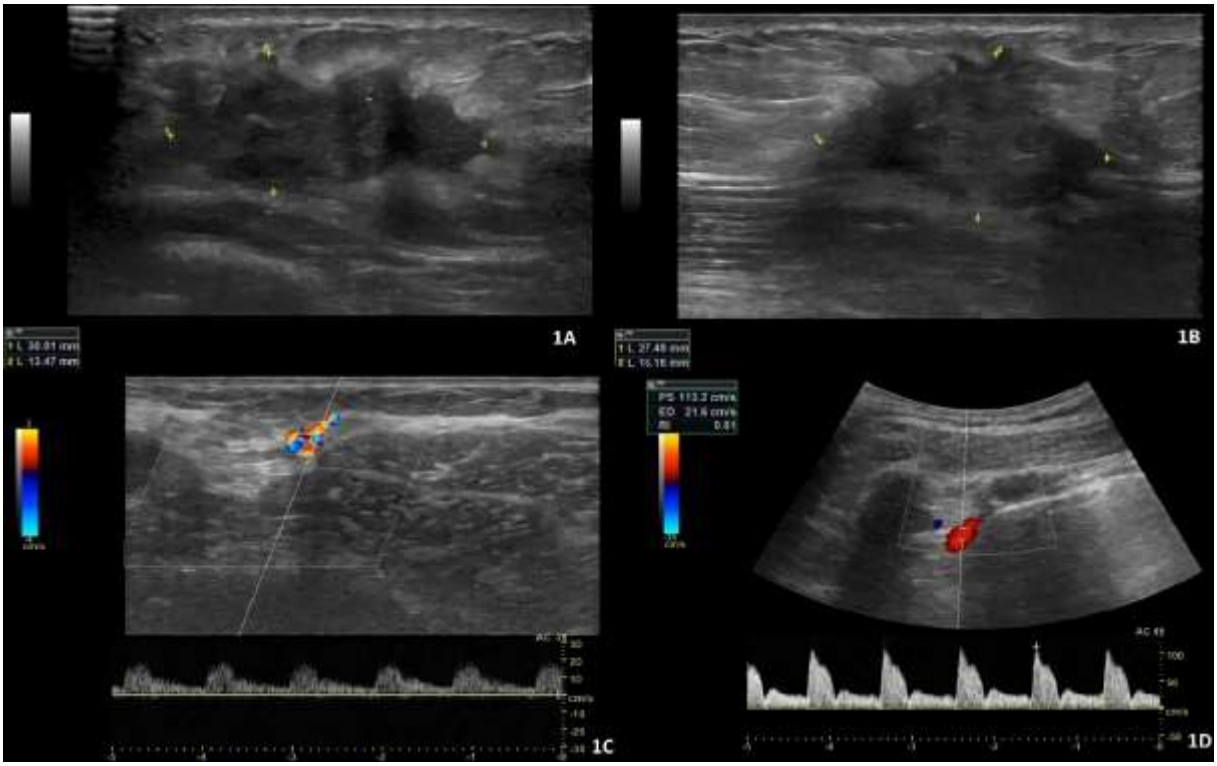

Figure 1:1A-1B An irregular shaped solid mass lesion in the upper outer quadrant of the right breast with a spicule contour, 30x27x15 mm in size, diagnosed as invasive ductal carcinoma. 1C:Evaluation of lateral thoracic artery by power doppler ultrasonography.1D: Evaluation of the internal thoracic artery by power doppler ultrasonography.

experience, and they were characterized using the Breast Imaging Reporting and Data System (BIRADS (American College of Radiology 2013)) classification system. The lesions in BIRADS categories 4 and 5 were lesions with a high probability of malignancy according to this classification. Location, size, lesion shape/margin features and echogenicity of the lesions were noted (Figure 1A-1B).

Biopsy Method

All lesions were sampled by US-guided core biopsy and pathological evaluation results were obtained for all lesions.

PDUS examination

Patients whose pathological examination results were presented as early-stage invasive breast carcinoma and who were decided to have an operation were performed an ultrasound examination before the operation. After the biopsy procedure, PDUS examination was performed 2-4 weeks later to prevent the flow parameter changes that may develop due to the procedure. PDUS imaging and spectral analysis were performed on the patient's ITA and LTA feeding the breast with lesions and the ITA and LTA feeding the healthy breast.

Patients were placed on their back with their hands under their heads and arms in flexion to detect LTA and ITA. The probe was first placed parallel to the edge of the pectoral muscle and LTA was detected with color doppler (Figure 1C). ITA was detected with color doppler by placing a probe in the second intercostal space of both sides (Figure 1D). ITA was located 1-2 cm lateral to the sternal border [16,17]. Spectrum examination in Doppler imaging was measured at the lowest pulse frequency repetition value that did not

Table 1. Patients demographic characteristics and lesion characteristics.

	Number	%
Age		
30-44	23	47,9
45-59	18	37,5
60 and above	7	14,6
Breast Pattern		
B	12	25,0
C	31	64,6
D	5	10,4
Breast Localization		
Right	19	39,6
Left	29	60,4
Lesion Localization		
Lower inner	10	20,8
Upper inner	8	16,7
Upper outer	15	31,3
Lower outer	7	14,6
Upper middle	8	16,7
Lesion Edge		
Spiculation	23	47,9
Angulation	11	22,9
Microlobulation	14	29,2
Lesion Shape		
Irregular	46	95,8
Oval	2	4,2

cause aliasing artifacts, the lowest Doppler inspection window, and a low wall filter (50 Hz). Peak systolic flow (PSF), peak diastolic flow (PDF), resistive index (RI) and Pulsatility index (PI) values were obtained for both arteries.

Statistical Analysis

Statistical analysis of all results was performed using the SPSS software version 22.0 (SPSS Inc., IBM Corp. Armonk, NY).

The Mann-Whitney U test was used for independent binary groups that did not fit the normal distribution. Categorical variables presented as ratio, continuous variables were presented as median (min-max) value and standart deviation (SD). A $p<0.05$ value was considered significant.

We used Reciever Operator Characteristics Curve (ROC) analysis to determine the effectiveness of the PDUS parameters and predict malignant breast lesions. Although the, LTA RI, LTA PSF, ITA PDF and ITA RI values were statistically significant in areas under the curve in the predict of malignant breast lesions and their specificities were high.

Results

The sociodemographic characteristics of the patients and the characteristics of the lesions are given in Table 1.

PSF, PDF, RI and PI values of LTA and ITA values were compared for the breast with the lesion diagnosed as invasive carcinoma and healthy breast.

LTA PSF, LTA RI and ITA RI values were found to be significantly higher in the invasive carcinoma side compared to the healthy side ($p<0.001$), and the ITA PDF value was significantly higher in the healthy side ($p<0.001$) (Table 2).

ROC Analysis Results

In deciding the diagnosis of invasive breast carcinoma, it was found that LTA PSF, LTA RI and ITA RI on the breast side diagnosed with carcinoma and ITA PDF values on the healthy breast side are very good diagnostic tests and the areas under the curve are significant. The values of the area under the curve (AUC) of these measurements are given in Table 3.

It has been determined that these values can be used to decide the distinction between invasive breast carcinoma and healthy breast. It was found that higher LTA PSF, LTA RI and ITA RI values in the breast with invasive breast carcinoma compared to the healthy

Table 2. Comparison of PDUS measurements of breast with malignant lesions and healthy breast*

	Control Breast			Invasive Carcinoma Detected Breast			
Measurement Value	Mean	S.D.	Median	Mean	S.D.	Median	p
LTA PSF	15,95	4,14	15,50	26,91	13,46	22,10	<0.001
LTA PDF	3,73	1,11	3,40	4,49	2,88	3,80	0.204
LTA RI	0,76	0,05	0,78	0,84	0,03	0,84	<0.001
LTA PI	2,03	0,35	2,11	2,11	0,18	2,15	0.323
ITA PSF	49,77	22,01	42,20	48,77	16,49	44,80	0.714
ITA PDF	10,68	3,77	9,40	6,95	3,59	6,40	<0.001
ITA RI	0,76	0,06	0,76	0,86	0,05	0,84	<0.001
ITA PI	3,10	0,57	3,22	2,99	0,55	3,22	0.336

LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow;
PDA: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index; SD: Standart Deviation
* Mann Whitney U

Table 3. ROC characteristics of various parameters

				% 95 Confidence Interval	
Variable	Area	Std. Error	p	Lower Bound	Upper Bound
LTA PSF	0,859	0,042	<0,001	0,777	0,942
LTA RI	0,944	0,027	<0,001	0,891	0,997
ITA PDF	0,784	0,048	<0,001	0,690	0,877
ITA RI	0,883	0,037	<0,001	0,811	0,956

LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow; PDA: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index

Table 4. Cut-off values and validity results

	Cut-off	Sensitivity	Spesifity	J statistic	LR+	LR-
LTA PSF	19,6	81,3	95,8	0,771	19,35	0,20
LTA RI	0,805	91,7	95,8	0,875	21,83	0,08
ITA PDF (low)	8,35	77,1	79,2	0,563	3,70	0,28
ITA RI	0,8	89,6	81,2	0,708	4,76	0,12

LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow; PDA: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index; LR: Likelihood ratio

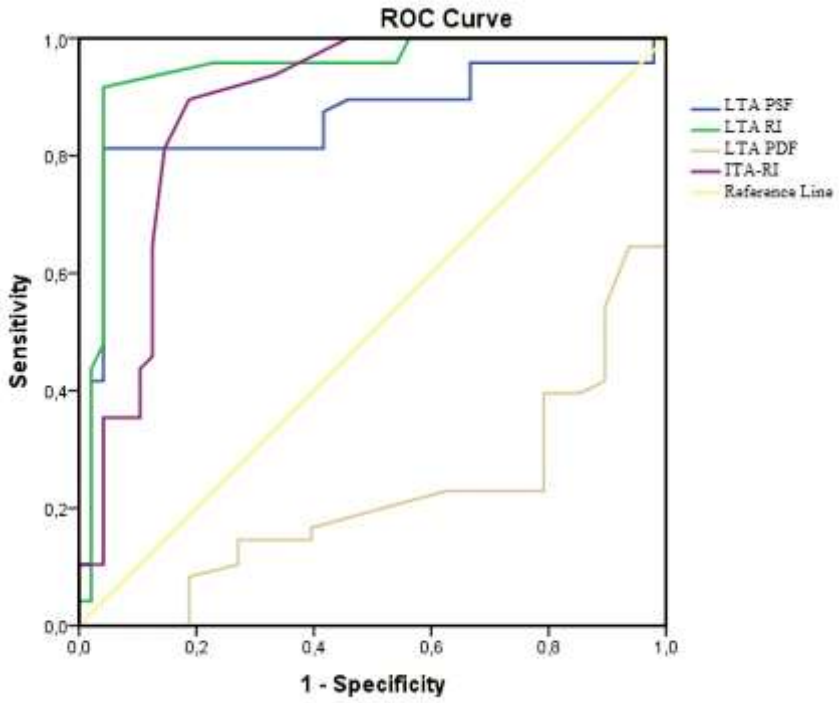

Figure 2: Area under curve in ROC analysis. LTA: Lateral thoracic artery, ITA: Internal thoracic artery; PSA: Peak systolic flow; PDF: Peak diastolic flow; RI: Resistive index; PI: Pulsatility Index

breast can predict the diagnosis of invasive breast carcinoma in the current breast. Low ITA PDF value in the healthy breast compared to the breast with invasive breast carcinoma can predict the diagnosis of invasive carcinoma in the other breast (Figure 2).

The optimal cut-off value for LTA PSF value is 19.6 cm/s, the sensitivity of this value is 81.3% and the specificity is 95.8%. If there are LTA PSF values above this cut-off value, that lesion can be interpreted in favor of a malignant lesion.

The optimal cut off value for LTA RI value was determined as 0.805, the sensitivity of this value was 91.7% and the specificity was 95.8%. The lesion detected at LTA RI values above this cut-off value can be interpreted in favor of invasive breast carcinoma (Table 4).

The sensitivity of the ITA PDF value below 8.35 for the detection of invasive breast carcinoma in the opposite breast is 77.1% and the specificity is 79.2%. Among these measurement values, the diagnostic value with the lowest false positivity and false negativity rates is the LTA RI value. The positive likelihood ratio value of this value is 21.83 and the negative likelihood ratio value is 0.008 (Table 4).

Discussion

According to the results of this study, from the quantitative data obtained in PDUS examinations of LTA and ITA arteries. The cut-off values of LTA PSA, RI and ITA RI values were determined to detect invasive breast carcinoma, and the sensitivity and specificity of these values are quite high.

Angiogenesis plays an important role in both local growth and distant metastases of breast cancer. Breast ultrasonography is an inexpensive diagnostic method that can be easily applied in young women with a family history, in patients admitted to outpatient clinics for reasons such as mastalgia, nipple discharge, and in women with palpable lesions on breast examination without radiation exposure [18]. In addition, it is more sensitive in detecting lesions that cannot be

detected in mammography in dense breasts [19].

The features of the masses detected in the breast such as their shape, contour features, and their location in the breast axis can be easily determined by ultrasonography. The characteristics of the masses can be determined by evaluating the vascularization of the masses with PDUS examination. In recent studies, it has been reported that PDUS examination is very important in increasing blood flow in the tumor and detecting neoangiogenesis and can be used to determine the malignant character of lesions [14,15].

It is known that new vascular structures that do not have a smooth muscle structure are observed among the malignant lesions detected in the breast [20]. Although it has been shown in various studies that the hypervascularity detected in US examination can provide information about the malignant character of the lesions. It has been stated that the RI and PI values detected in the lesions are not good diagnostic tools for lesion characterization [21]. In a recent study, it was shown that the diastolic flow reversed in the flow in the mass detected in the breast and the RI value above 1 was most likely associated with lesion malignancy [21]. However, it has been previously stated that lesion vascularization is not always evident in patients with breast cancer. Accordingly, PDUS evaluation can gain sensitivity when evaluated together with the morphological features of the mass identified in ultrasonography [22]. Although PDUS imaging is thought to be helpful in distinguishing benign and malignant solid masses, it has been stated that it does not have high predictive quantitative values. PDUS imaging can only be used to support the pre-diagnosis of lesions with suspicious morphological features in B-mode US examination [23].

The hypothesis of our study was that vascularization could change in the breast developing malignancy rather than lesion vascularization for malignancy characterization. Therefore, PDUS examination of the main vascular structures feeding the breast was performed and it was aimed to obtain quantitative data. In some previous studies investigating the value of LTA and axillary artery blood flow in determining the lesion characteristics in breast masses, have been conducted. It has been reported that a LTA RI value of 0.67 and above is significant for detecting malignancy in these studies [17,24]. However, even in the healthy breast determined as the control group in our study, LTA values were generally above 0.67. This explains why the sensitivity and specificity values of the values determined in our study are high. In addition, the statistical analyzes made in our study are more comprehensive and powerful. Moreover, as far as we know, there is no current publication where ITA evaluations were made, and in the results of our study, not only LTA RI but also LTA PSA and ITA RI values were obtained with high sensitivity and specificity predicting malignancy. This also supports our hypothesis. In addition, unlike other studies, this study allows for easily calculable evaluations rather than spectral examinations such as negative diastolic flow, which are rare and not always possible to detect.

According to these data, LTA RI, LTA PSF, ITA PDF and ITA RI values may be a new diagnostic method with high sensitivity and specificity that can be used for the detection of invasive breast carcinoma.

The strength of this study is that it is prospective. Another strong feature is that multiple parameters belonging to LTA and ITA arteries work together with their diagnostic features and obtain cut-off values with high sensitivity and specificity values.

This study had some limitations. First of all, the study population was small, because the patients included in this study consisted of

patients who were made surgical preparations immediately after their diagnosis, and patients diagnosed with locally advanced breast cancer who were diagnosed with neoadjuvant chemotherapy in our center were excluded. It was thought that the application of neo-adjuvant chemotherapy would affect breast vascularization, and measurements were not taken from these patients before the operation. Second, the evaluations of the patients were made only for lesions diagnosed with pathologically invasive breast carcinoma. Further studies with larger populations including vascular changes of benign lesions can be conducted.

Conclusion:

In conclusion, this study presents new quantitative diagnostic tests with high sensitivity and specificity, easily accessible, and applicable in detecting breast cancer.

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Original Article

Frequency of Incidental Pulmonary Findings Detected on PET/CT Images of Elderly Patients Diagnosed with Extrapulmonary Malignant Neoplasm

Ekstrapulmoner Malign Neoplazm Tanılı Yaşlı Hastaların PET/BT Görüntülerinde İnsidental Pulmoner Bulguların Sıklığı

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ABSTRACT

Introduction: Reporting thorax imaging findings on 18F-fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET/CT) is important for patient management. Even if some pulmonary findings are benign, they can have serious life-threatening consequences. This study aimed to investigate the frequency of benign or malignant pulmonary findings, which were simultaneously detected in PET/CT scans, of elderly patients with extrapulmonary malignant neoplasms.

Methods: Patients aged ≥65 years, applying to nuclear medicine department of a tertiary level health unit between November 2017 and April 2018 were retrospectively evaluated. Demographic and clinical information and PET/CT scans were obtained from their previous hospital records. Data obtained were analyzed using the SPSS version 22.

Results: A total of 112 patients (mean age, 72.8 ± 7.0; females, 58.9%) were included in the study. In total, 38.4% of the patients had a smoking history, and 39.2% were exposed to second-hand smoke. The most common indications for PET/CT imaging were post-treatment evaluation (42.9%) and staging (35.7%). Predominantly diagnosed malignancies were cancers of the gastrointestinal system (26.8%), breast (26.8%), and urogenital system (17%). While most patients had benign or malign pulmonary findings in thoracic images, no abnormal pulmonary findings were observed in only 24 patients (21.4%). The most common findings were emphysema (39.3%), metastatic nodules (27.7%), bronchial wall calcifications (14.3%), and air trapping/cysts (9.8%).

Discussion and Conclusion: This study revealed that 78.6% of elderly patients with extrapulmonary malignant neoplasms undergoing PET/CT scans had at least one pathologic lung finding. Although most of these findings are benign, reporting of them is important in the management and clinical outcomes of patients with malignancy.

Keywords: Elderly patient, PET/CT, malignant neoplasm, incidental pulmoner findings

ÖZET

Giriş ve Amaç: 18F-fluorodeoksiglukoz (18F-FDG) pozitron emisyon tomografi-bilgisayarlı tomografide (PET/BT) malignite kuşkulu lezyonlara ilaveten, benign karakterdeki bulguların raporlanması da hasta yönetiminde önemlidir. Bazı pulmoner bulgular benign olsa bile hayati tehlike oluşturan ciddi sonuçlara yol açabilir. Bu çalışmanın amacı, ekstrapulmoner malign neoplazm tanısı ile PET/BT çekilmiş olan yaşlı hastalarda benign ya da malign pulmoner bulguların sıklığını araştırmaktır.

Yöntem ve Gereçler: Üçüncü basamak sağlık kuruluşu nükleer tıp bölümüne Kasım 2017-Nisan 2018 tarihleri arasında başvuran 65 yaş ve üzeri hastalar retrospektif değerlendirildi. Demografik verileri, klinik bilgileri ve PET/BT görüntüleri hastane kayıtlarından elde edildi. Bulgular SPSS 22 kullanılarak analiz edildi.

Bulgular: Toplam 112 hasta (yaş ortalaması 72.8±7.0; %58.9'u kadın olan) çalışmaya dahil edildi. Hastaların %38.4'ü aktif, %39.2'si pasif sigara içicisiydi. En sık PET/BT çekimi endikasyonu, tedavi sonrası yanıt değerlendirme (%42.9) ve evreleme (%35.7) idi. Tanıların çoğunluğunu gastrointestinal

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sistem (%26.8), meme (%26.8) ve ürogenital sistem (%17) maligniteleri oluşturmaktaydı. Hastaların çoğunda toraks kesitlerinde benign ya da malign pulmoner bulgulara rastlanırken, sadece 24 hastada (%21.4) hiçbir anormal pulmoner bulgu izlenmedi. En sık saptanan bulgular; amfizem (%39.3), metastatik nodül (%27.7), bronş duvarı kalsifikasyonu (%14.3) ve hava hapsi/kisti (%9.8) idi.
Tartışma ve Sonuç: Bu çalışma, ekstrapulmoner malign neoplazm tanısı ile PET/BT çekilen yaşlı hastaların %78.6'sında en az bir anormal pulmoner bulgunun olduğunu gösterdi. Bu bulguların çoğu iyi huylu olmakla birlikte, bunların raporlanması malignitesi olan hastaların yönetimi ve klinik gidişatında önemlidir.

Anahtar Kelimeler: Yaşlı hasta, PET/BT, malign neoplazm, insidental akciğer bulguları

Introduction

18F-fluorodeoxyglucose (18F-FDG) positron emission tomography/computed tomography (PET/CT) is a valuable imaging modality commonly for early detection, accurate staging, and evaluating treatment response of many cancers. Its main advantage in relation to other radiological imaging methods is the availability of functional imaging [1]. Oncologic PET/CT scans can be used to view cross-sectional tomographic and functional images from the vertex to the proximal thigh. Besides its ability to detect malignancies, PET/CT also reveals non-malignant findings.

The incidence of malignant diseases is increasing due to several factors such as advanced age, increased smoking rate, genetic factors, poor nutrition, air pollution, and occupational exposure. Imaging techniques exert an important role in the management of patients with malignant diseases. Among them, PET/CT has gained relevance not only for diagnosis but also for staging and treatment outcome evaluation. The rate of incidental findings has increased along with the use of low dose CT, and the importance of non-malignant incidental findings for patient management is well established among clinicians [2]. Therefore, during clinical evaluation it is crucial to carefully examine non-diagnostic CT images in the oncologic PET/CT scans [3]. It is recommended that such additional findings should be taken into account, as they are important for disease management and prognosis [4].

In the elderly patients, it is crucial to distinguish between age-related and pathological findings to avoid misdiagnoses [5]. Therefore, special knowledge on diagnostic imaging of elderly patients is required [6].

The aging causes reduced lung elasticity due to the loss of supportive tissue. Homogeneous airway dilatation may occur in the absence of inflammation, fibrosis, alveolar wall destruction or distortion [7,8]. Emphysematous and fibrotic changes in the basal segments of lung parenchyma are commonly seen, as well as morphological changes such as progressive calcifications of the airways and thorax [7-9].

A previous study reported that the CT images of elderly (> 65 years) showed a significant number of asymptomatic emphysema than those of young adults (<55 years). The same study found that 60% of elderly adults exhibited interstitial changes of the subpleural reticular pattern [10]. Another study reported that asymptomatic air cysts were frequently observed in elderly patients [11]. A study showed that asymptomatic older patients had greater prevalence of air trapping and extensive degree of air trapping also correlated with aging [12].

The aim of the present study was to evaluate pulmonary findings of elderly patients undergoing PET/CT scans due to extra-pulmonary malignant neoplasms. The results will also provide an overview on the non-

malignant uses of PET/CT in elderly population.

Materials and Method

Setting and participants

The PET/CT scan of cancer patients sent to the department of nuclear medicine at a tertiary care unit in Turkey was retrospectively analyzed. The institutional ethical committee approved this study.

Demographic (i.e., age, gender, smoking status, comorbidities) and clinical (i.e., diagnosis, PET/CT indications) information were obtained from the hospital's database and the PET records/CT informed consent forms. Patients aged <65 years and diagnosed with lung cancer were excluded from the study.

The study was approved by the Recep Tayyip Erdoğan University of Local Ethics Committee (protocol number: 2018/109), and Helsinki declaration principles were followed.

18F-FDG PET/CT scan

All patients fasted for at least 6 hours before PET/CT scans. For all patients, information about the PET/CT scanning indication, clinical history, chemotherapy and radio-therapy history, height and weight, and fasting blood glucose levels were recorded before 18F-FDG injection. All patients had a fasting and blood glucose level was <200 mg/dL before imaging. The approximately 220–370 MBq 18F-FDG was intravenously administered to each patient. Following a resting period of approximately 50–60 min in the waiting room, the patients were taken for the PET/CT scan (by Siemens Biograph mCT, 20 excel). Images were acquired from the vertex to the upper thigh. CT images were taken with a 5 mm slice thickness and an average of 120-kVp/100-mAs dose range without intravenous contrast. PET images were acquired within 2–4 min per bed. Both the PET and CT images were obtained during normal tidal breathing.PET images were reconstructed using CT for attenuation correction. PET, CT, and fused PET/CT images displayed as coronal, sagittal, and transaxial planes were viewed on a syngo-via workstation (Siemens Healthineers). A nuclear medicine physician interpreted all images. All incidental lung findings detected on the PET/CT images were reported.

Statistical analysis

All statistical analyses were performed using the SPSS version 22. Descriptive statistical methods were used for demographic, clinical, and radiological features. Categorical variables were analyzed using the Pearson's Chi-square test, whereas continuous variables were analyzed using the student's t-test. A level of $p<0.05$ was considered statistically significant.

Results

A total of 112 patients were evaluated. The mean age was 72.8±7 and 58.9% were females. The proportion of current and past smokers was 38.4%, and 24% reported second-hand smoke exposure. A total of 76.8% of the patients had at least one comorbid disease, including hypertension (65.2%), diabetes mellitus (14.3%), and coronary artery disease (11.6%). The patients' demographic data is presented in Table 1.

The patients were referred from the medical oncology (51.8%), general surgery (13.4%), hematology (12.5%), radiation oncology (9.8%) departments respectively for PET/CT scan.

Most patients undergoing PET/CT scan had gastrointestinal malignancies (26.8%), breast cancer (26.8%), and urogenital malignancies (17%). The proportion of patients with chemotherapy and radiotherapy histories was 63.4%and 25.8%, respectively. PET/CT scan indications included staging (35.7%), post-

Table 1. Demographic characteristics of all patients

Variable	n (%)
Age (mean, ±SD)	72.8 ± 7
Gender	
Female	66 (58.9)
Male	46 (41.2)
Smoking status	
Current/former smoker	43 (38.4)
Presence of second hand smoke exposure	27 (24.1)
Presence of comorbid disease	86 (76.8)
Presence of obstructive lung disease	21 (18.8)
Presence of inhaler medication use	22 (19.6)
Presence of tuberculosis or pneumonia history	11 (9.8)

Table 2. Pulmonary findings of the study population

Pulmonary findings	n (%)
Emphysema	44 (39.3)
Metastasis	31 (27.7)
Bronchial wall calcification	16 (14.3)
Air trapping/Cyst	11 (9.8)
Calcific nodule	10 (8.9)
Sequela fibrotic changes in apex	10 (8.9)
Bilateral pleural effusion	4 (3.6)
Reticular/Reticulonodular infiltration	6 (5.4)
Pleural thickening	5 (4.5)
Solitary pulmonary nodule	3 (2.7)
Calcific pleural thickening	2 (1.8)
Subsegment atelectasis	3 (2.7)
Bronchiectasis	1 (0.9)
Collapse and consolidation	1 (0.9)
Ground glass opacity	1 (0.9)

treatment evaluation (42.9%), and re-staging (21.4%) respectively.

Pathologic lung imaging findings were observed in 78.6% of the patients. The most common findings were emphysema (39.3%), metastatic nodular lesion (27.7%), and bronchial wall calcification (14.3%). Emphysema and metastatic lung nodules are shown in Figure 1, 2 and collapse-consolidation image determined by PET/CT for evaluation of treatment response after chemotherapy is given in Figure 3.

Table 3. Comparison of the variables according to patients' smoking status

	Current/former smoker (n:43)	Non-smoker (n:69)	p
Gender male	38 (88.4%)	8 (11.6%)	<0.001
Presence of obstructive pulmonary disease*	9 (20.9%)	12 (17.4%)	>0.05
Incidental pulmonary findings	41 (95.3%)	47 (68.1%)	<0.001
History of tuberculosis and pneumonia	5 (11.6%)	6 (8.7%)	>0.05

*Asthma+ COPD patients

Information about pulmonary findings is presented in Table 2.

A total of 43 patients (38.4%) had current/ former smoking history and most were male (88.4%). Most of the smokers (95.3%) had pathologic lung findings (p <0.005). Table 3 shows the patient characteristics according to their smoking status.

Among the 112 patients, 21 of them were diagnosed with obstructive pulmonary disease, 11 patients had chronic obstructive pulmonary disease (COPD) (52.4%), and 10 patients had asthma (47.6%). The rate of current/former smokers was 20.9% of the total but it was 72.8% among patients with COPD.

Lung metastases were most commonly caused by breast (29%) and colon cancers (22%). Other cancers associated with lung metastasis were bladder cancer (6%), renal cell carcinoma (6%), endometrium carcinoma (6%), cancers of undifferentiated origin (6%), gastric cancer (3%), larynx cancer (3%), pancreatic cancer (3%), liver cancer (3%), sarcoma (3%), and multiple myeloma (3%) respectively.

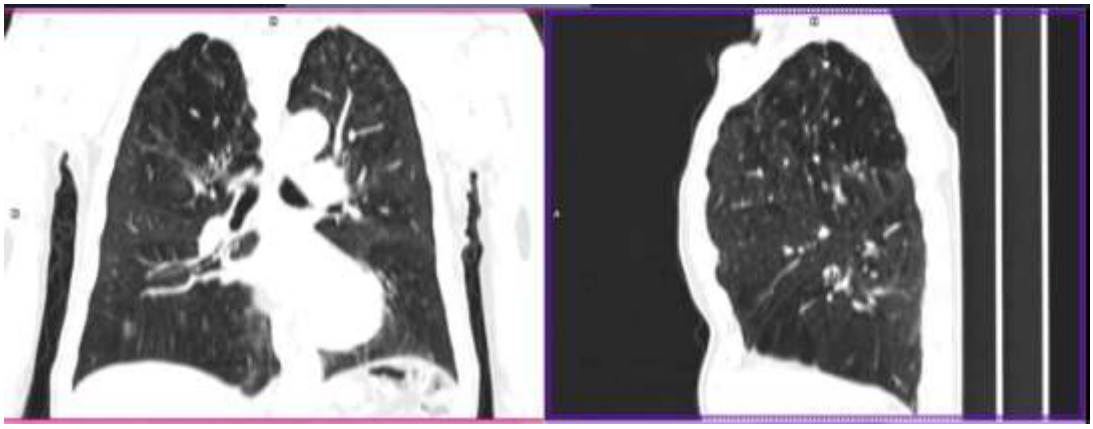

Figure 1. Emphysematous lung image in coronal and sagittal sections on CT

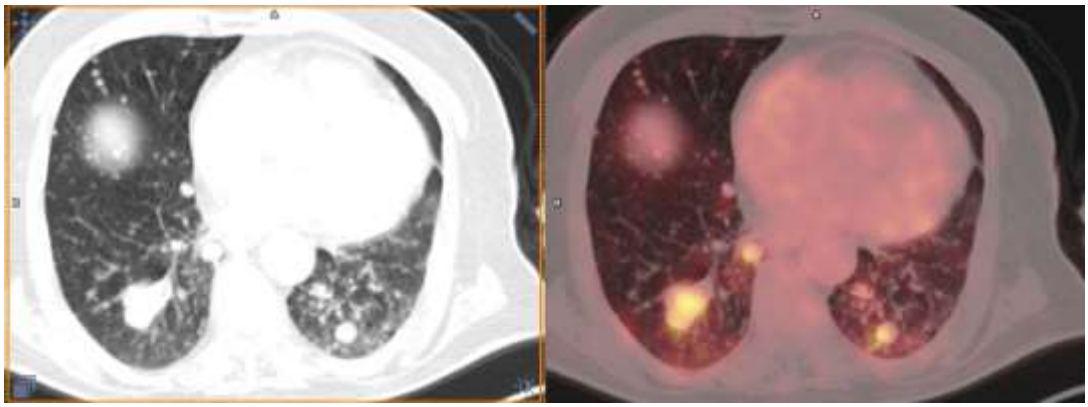

Figure 2. Transaxial CT (a) and fusion image (b) on PET/CT scan showed multiple lung metastases in a 64-year-old male patient diagnosed with a rectal cancer

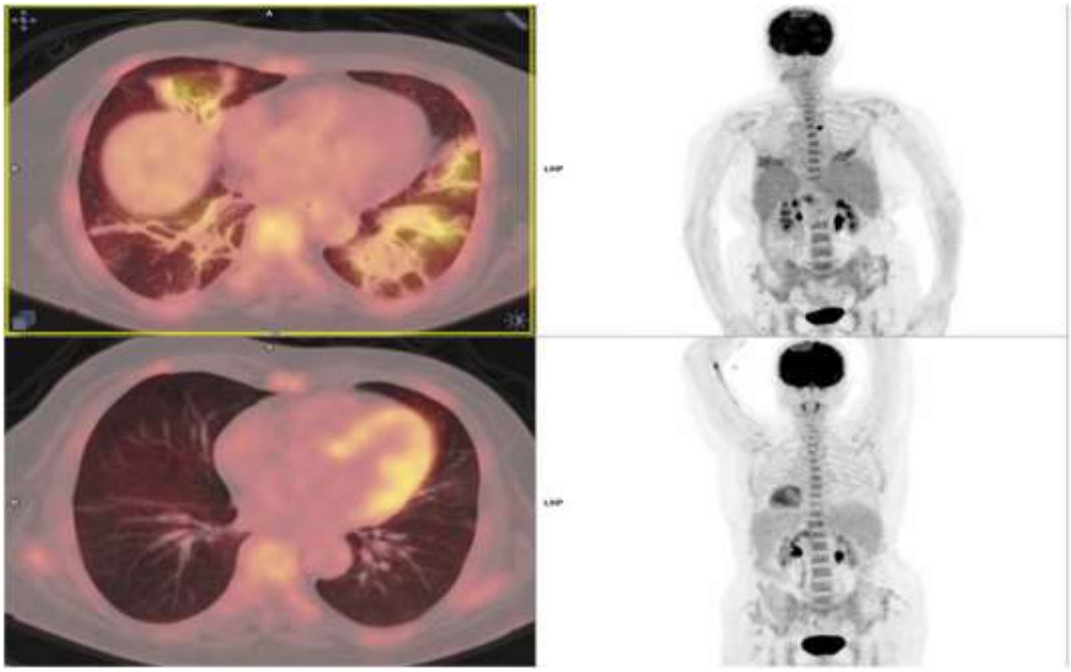

Figure 3. Transaxial fusion PET/CT (a, b) and maximum intensity projection (c, d) images showed post-chemotherapy collapse and consolidation (a, c) in a 77-year-old male patient diagnosed with a hematologic malignancy

Discussion

In the present study, thoracic CT images of 112 elderly patients undergoing PET/CT scans were retrospectively examined. Among all patients, only 24 of them (21.4%) had no pathologic lung findings on thoracic CT images. The most common pathologic findings were emphysema (39.3%), metastatic lung nodules (27.7%), bronchial wall calcifications (14.3%), and air trapping/air cysts (9.8%). Other findings included calcific nodules, sequelae fibrotic changes in the apex, subpleural reticular/ reticulonodular infiltration, and solitary pulmonary nodules. Calcific pleural thickening, subsegmental atelectasis, bronchiectasis, collapse/ consolidation, and ground glass opacity were less commonly found. All these findings significantly more frequent in the patients with a smoking history ($p < 0.001$).

It is known that with aging, distal airspaces expand due to the loss of supportive tissue, resulting in changes designated as "senile lung, senile hyperinflation, or senile emphysema". Homogeneous airway dilatations can be observed in elderly patients in the absence of inflammation, fibrosis or other structural pathologies [7]. A study comparing the radiological findings in the lung parenchyma of elderly (>75 years) and younger individuals (<55 years), found a higher rate of centrilobular emphysematous changes in the elderly group. The same study found a 60% rate of interstitial changes with subpleural reticular pattern among elderly individuals [10]. Another study showed that 25% of asymptomatic elderly patients presented small cystic lesions [11]. In the present study, the most common incidental lung finding was emphysema, which is consistent with that observed in the existing literature (39.3%). However, the rate of interstitial changes associated with the subpleural reticular pattern was only 4.5%, which may have been due to the CT image technique used. In the present study, CT images were acquired at an average of 120-kVp and 100-mAs dose, in 4-mm section thickness and in inspiratory or expiratory phases instead of 120-kVp and 400-mAs dose, 1-mm section thickness, thin-section CT. This may have caused the inconsistency between the results of the present study and those of the existing literature [11,12]. In the present study, the rate of air cysts was 9.8%, whereas previous studies report a rate of 25% [11]. This discrepancy may have also been caused by the used image section thickness.

Lung is one of the most common sites for primary and metastatic malignancies and a challenging site to diagnose primary versus a metastatic origin of the tumor on cytology. The developments in CT technology have led to an increasing detection of pulmonary nodules in thorax CT. A previous study reported that 20% of the patients with extra-pulmonary malignant neoplasm presented metastatic lung nodules on thorax CT images [13]. In the latter study, thorax images were evaluated using thin-section CT (section thickness, 1 mm). The detection of incidental pulmonary nodules has increased with the use of thin-section thorax CT [14]. In our study, metastatic lung nodules were detected in 27.7% of the cases, although images were not acquired using thin-section CT (section thickness, 4 mm). This ratio is relatively high compared with that reported in the literature. In our department, PET/CT images have a 5-mm thickness and thus have low sensitivity for nodules <4 mm. Therefore, there is a possibility for small nodules to go undetected. However, histopathological sampling of the detected lung nodules was not performed. The presence of multiple nodules on PET/CT images and FDG uptake levels of nodules are findings that support the occurrence of metastases. Since most available nodules present these features, they have been

clinically accepted as metastases. The clinical history of a known extra-pulmonary primary and the radiologic findings of multiple nodules in the lung are useful in arriving at the right diagnosis but is not always reliable. The approach to the diagnosis of metastatic tumors in the lung on cytology should be largely guided by the previous clinical history and comparison with previous tissue/cellular material if available [15]. Some nodules classified as malignant may, in fact, be benign, which helps to explain our findings of a higher rate of lung metastases compared with those reported by previous studies.

When the high occurrence of lung metastasis is analyzed from a different perspective, it becomes questionable whether cancer diagnosis is delayed in elderly patients. In the previous literature, 20% of lung metastases were detected without discriminating between old and young patients [13]. Pulmonary metastases are hematogenous distant metastases. In the present study, a higher rate of metastatic lung nodules than that reported in the previous literature was found, which may be related to the fact that elderly patients are diagnosed with advanced stage cancers. Comorbidities in elderly patients may overshadow cancer-related symptoms, thereby delaying cancer diagnosis. Therefore, a careful evaluation of geriatric patients should be performed, excluding malignancies in the differential diagnosis.

According to the previous literature, lung metastases were most commonly caused by breast cancer, followed by colon cancer [16]. Accordingly, in the present study the most common causes of lung metastasis were breast (29%) and colon cancers (22%).

Among the patients analyzed in the present study, only 21.4% had normal thorax CT images. Most patients had one or more pathological findings. Beside the metastatic malignant lesions, reporting other incidental lung findings such as emphysema, collapse/ consolidation, bronchiectasis, pleural effusion, and interstitial pattern can contribute to improve the patients' quality of life through administration of adequate treatment and interventions. Such findings are therefore important for the subsequent management of cancer patients.

Conclusion

The prevalence of the geriatric patient population is increasing nationally and worldwide, leading to a higher cancer incidence. The present study investigated thorax images obtained from PET/CT scans of the geriatric patient population with extrapulmonary malignant neoplasms. Most patients (78.6%) had one or more pathologic lung findings. Consistent with the previous literature, our most frequent finding was emphysema. We found lung metastasis as the second most common finding, which contradicts previous studies. Although the interstitial changes are reported with a high rate in the existing literature, our study found a low rate, which was considered to be caused by the imaging procedures' differences.

CT scans of the lung parenchyma show common findings in elderly patients, which are thought to be related with collagen changes. The differential diagnosis between aging-related imaging findings and those secondary to disease is difficult and can not be achieved using imaging studies alone. These lesions should be compared with previous examinations and follow-up [7,10,17-19]. Therefore, the reporting of incidental findings in the geriatric population diagnosed with extrapulmonary malignancy may contribute to improve patient management.

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Original Article

The Predictive Value of FDG-PET / CT in Assessing Bone Marrow Involvement in Hodgkin Lymphoma Patients; A Single Center Experience

Hodgkin Lenfoma Hastalarında Kemik İliği Tutulumunu Değerlendirmede FDG-PET/BT'nin Prediktif Değeri: Tek Merkez Deneyimi

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ABSTRACT

Purpose: This study was conducted to determine the predictive value of positron emission tomography (PET/CT) used in staging Hodgkin Lymphoma (HL) at the time of diagnosis in determining bone marrow (BM) involvement.

Material and method: The patients diagnosed with Hodgkin lymphoma in our hematology department between 2009-2019 were analyzed retrospectively. The study included a total of 46 patients who underwent both BM biopsy and PET/CT for staging at the time of diagnosis.

Results: The mean age of the 46 patients was 40 years (19-80). BM involvement was determined in three patients from BM biopsy and in 14 patients from PET/CT performed at the time of diagnosis. When PET/CT results were analyzed according to BM biopsy results, it was found that the sensitivity was 100% (3/3) and the specificity was 74.4% (32/43).

Conclusion: The sensitivity of PET/CT is very high in detecting BM involvement in HL patients, and is a non-invasive test. However, in doubtful cases it may be more appropriate to perform a BM biopsy, even though it is invasive, as PET/CT may cause false positive patient staging due to its low specificity.

Keywords: Hodgkin lymphoma, bone marrow involvement, PET/CT

ÖZET

Giriş ve Amaç: Hodgkin Lenfoma hastalarında tanı anında evrelemede kullanılan PET/BT tetkikinin kemik iliği tutulumunu belirlemedeki yerini saptamak

Yöntem ve Gereçler: Sağlık Bilimleri Üniversitesi Ankara Dışkapı Yıldırım Beyazıt Eğitim ve Araştırma Hastanesi Hematoloji kliniğinde 2009-2019 yılları arasında takip edilen Hodgkin lenfoma tanılı hastaların dosyaları retrospektif olarak incelendi. Bu hastalar arasında tanı anında hem kemik iliği biyopsisi yapılan, hem de PET/BT çekilen toplam 46 hasta çalışmaya dahil edildi.

Bulgular: Toplam 46 hastanın yaş ortalaması 40 (19-80) idi. Hastaların kemik iliği tutulum durumları incelendiğinde kemik iliği biyopsisinde üç hastada tutulum saptanırken, tanı anında yapılan PET/BT'de 14 hastada tutulum olduğu görülmüştür. PET/BT sonuçlarının kemik iliği biyopsi sonuçlarına göre değerlendirildiğinde duyarlılığının %100 (3/3), özgüllüğünün ise %74,4 (32/43) olduğu görüldü.

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Tartışma ve Sonuç: Hodgkin lenfoma hastalarında kemik iliği tutulumunu saptamada non invaziv bir test olan PET/BT'nin duyarlılığı çok yüksektir. Ancak özgüllüğünün düşük olması sebebi ile yanlış pozitif hasta evrelemesine sebep olabileceğinden şüphe halinde invaziv de olsa kemik iliği biyopsisi yapılması daha uygun olacaktır.

Anahtar Kelimeler: Hodgkin Lenfoma, kemik iliği tutulumu, PET/BT

Introduction

Hodgkin lymphoma (HL) is a lymphoid malignancy originating from B cells [1], which accounts for approximately 12% of all lymphomas [2]. In histomorphological classification it is divided into two major groups; classical type and nodular lymphocyte predominant (NLP) type. Classical type HL includes nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich (LR) and lymphocyte depleted (LD) subtypes [1].

Approximately 90% of cases are classical type HL patients. NS type is the most common subtype of classical HL. Although the average age at diagnosis varies between 20 and 34 years, differences can be detected between ethnic groups. It is generally seen more frequently in males than females. As with most other hematological malignancies, one of the most important prognostic markers in HL patients is stage. Correct staging of patients at the time of diagnosis plays a major role in the future management of patients. The Ann-Arbor classification with Cotswold modification is still used for staging HL [3].

With Cotswold modification, liver biopsy and laparotomy are removed from the routine staging of HL patients, while another invasive procedure, bone marrow (BM) biopsy, is still performed for staging [4]. Stage I-II is defined as early stage and stage III-IV as advanced stage disease [5]. Even if BM involvement exists alone, it advances the stage and therefore has significant impact on the treatment and prognosis [6].

BM involvement is an indicator of generalized disease since these cases are regarded as stage IV regardless of lymph node involvement. Although BM biopsy has been accepted as the gold standard to detect the involvement of BM since the 1970s, it is an invasive procedure and can cause some serious complications [4].

However, fluorodeoxyglucose (FDG) PET/CT has recently been the subject of research on the topic of determining BM involvement [6]. In the last 20 years, FDG / PET-CT has been used extensively for staging lymphomas. Several studies have retro-spectively investigated the use of PET-CT to detect BM involvement, and have reported that FDG/PET-CT showed superior sensitivity compared to BM biopsy. Of these studies, Tzu-Hua et al[7] showed the sensitivity of PET / CT to be 96.8%, whereas the sensitivity of BM biopsy was 32.3%. In another study, Weiler-Sagie et al[8] reported PET-CT sensitivity as 97%, and the sensitivity of BM biopsy as 15%. According to the results of the German Hodgkin working group, PET-CT performed at the time of diagnosis was reported to describe the involvement of BM with high sensitivity proved by BM biopsy. With excellent negative predictive value, PET is a very accurate and reliable tool for excluding BM involvement. [4].

The aim of this study was to determine the role of PET/CT examination used in staging at the time of diagnosis in determining BM involvement in HL patients diagnosed in our center.

Patients and methods

A retrospective evaluation was made of patients with HL diagnosed in the Hematology

Table 1. Demographic and clinical features of patients

Variable (N=46)		(n, %)
Disease subtype		
	LR	4 (8.7)
	MC	11 (23.9)
	NLP	7 (15.2)
	NS	24 (52.2)
Age at diagnosis (median, years)		40.0 [19.0-80.0]
Gender		
	Male	25 (54.3)
	Female	21 (45.7)
Stage at diagnosis		
	I	2 (4.3)
	II	26 (56.6)
	III	14 (30.4)
	IV	4 (8.7)

LR: Lymphocyte rich, MC:Mixed cellularity, NLP:Nodular lymphocyte predominant, NS:Nodular sclerosis

Clinic of the University of Health Sciences Ankara Diskapı Yildirim Beyazit Training and Research Hospital in the period 2009-2019. Of these patients, 46 who underwent both BM biopsy and PET/CT at the time of diagnosis were included in the study. Patients who had only BM biopsy or PET-CT at the time of diagnosis were excluded from the study. Age, gender, HL type and subtype, and disease stage were recorded. BM biopsy was considered the gold standard for the detection of involvement.

Statistical analysis

Study data were analyzed using SPSS 24 software. Descriptive statistics and frequency tables were used. Descriptive data were given as a percentage. Negative predictive and positive predictive values, sensitivity and specificity values were calculated.

Ethical approval and informed consent

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. As a standard of care/action of our hospital, the patient records confirmed that all the study patients gave informed consent at the time of hospitalization and before the administration of any intervention. The study was approved by University of Health Sciences Turkey, Ankara Dışkapı Yıldırım Beyazit Training and Research Hospital Ethics Committee (protocol no: 90/05, date: 22.06.2020).

Results

Of the 46 HL patients included in the study, 25 (54.3%) were male and 21 (45.7%) were female. The median age at diagnosis was 40.0 years (19.0-80.0). 84.8% of the patients were classical type HL and 15.2% were NLP type HL. The most common subtype in patients with classical type HL was NS in 24 (52.2%) patients. Other subtypes were MC in 11 (23.9%) patients and LR in four (8.7%) patients. No patient with LD subtype was detected. At the time of diagnosis, 28 (60.9%) patients were early stage (stage I-II), and 18 (39.1%) were advanced stage (stage III-IV). The demographic and clinical features of the patients are given in Table 1. When the BM involvement status of the patients was examined, involvement was detected in three patients in BM biopsy, and PET/CT showed increased uptake of FDG in 14 patients, demonstrating involvement at the time of diagnosis. BM involvement rates according to the diagnostic methods are given in Table 2. As a result of the analyses made by comparing the results according to the results of BM biopsy, the sensitivity of the PET / CT method in terms of BM involvement was 100% (3/3) and specificity was 74.4% (32/43) (Table 3).

Discussion

All HL patients should be staged during diagnosis to determine the appropriate treatment protocol and prognosis. History, physical examination, radiological imaging, and BM biopsy are part of the routine staging

Table 2. Bone marrow involvement rates according to diagnostic methods

(N=46)		Bone marrow involvement in bone marrow biopsy		Total
		(+) (n)	(-) (n)	
Bone marrow involvement in PET/CT	(+) (n)	3	11	14
	(-) (n)	0	32	32
Total		3	43	46

Table 3. PET/CT sensitivity, specificity and positive-negative predictive values

	Value	%95 confidence interval
Sensitivity	%100	29.24-100
Spesificity	%74.42	58.83–86.48
Positive predictive value	%21.43	14.08–31.23
Negative predictive value	%100	

process [9, 10]. Correct staging is important to be able to determine the appropriate treatment protocol. In addition, it is important in terms of side-effects and reducing toxicity [11]. BM involvement in HL patients is compatible with stage 4 disease. Although BM involvement rates vary according to the developmental level of countries, it is seen in approximately 10% of the adult population. [9, 10] In the current study, BM involvement was observed in three patients (6.5%) according to the histopathological findings.

BM biopsy is an invasive procedure used to diagnose a large number of hematological diseases and to evaluate treatment response. The posterior iliac crest is considered the most suitable region for biopsy. The frequency of complications is very low during and after this procedure (0.05%). The most common complication is bleeding.[12] Other complications can be listed as local infection at the biopsy site and needle breakage during the procedure. More rarely, transient neuropathy accompanied by gluteal compartment syndrome secondary to bleeding at the biopsy site may occur. In addition, patients with osteoporosis or osteomyelitis are at risk of bone fracture at the biopsy site. [12-14]

Since the procedure is invasive and there is a slight risk of the complications mentioned above, other methods that can be used instead of this procedure have been the subject of research. The first of these is the PET/CT method, which is currently used very often for staging. PET/CT is widely used in the initial staging of patients diagnosed with lymphoma and in evaluating the response to treatment. [11] In addition, it has high sensitivity and specificity in detecting extranodal involvement.[6] In this study, in which it was

aimed to determine the potential role of PET CT in detecting BM involvement at the time of diagnosis in HL patients, the negative predictive value and sensitivity of PET-CT was 100% and specificity was 74%. Eser A. et al.[15] analysed 104 patients with HL and the negative predictive value and sensitivity of PET-CT was reported to be 100% and specificity was 68.9%. In a study by Muzahir S. et al. [11] 122 patients diagnosed with HL were evaluated. The sensitivity of PET-CT was reported as 100%, specificity as 76.5% and the negative predictive value was 76.5% in terms of BM involvement.

Considering the data of the current study and other data in the literature, it can be seen that the sensitivity of PET / CT is quite high but the specificity is relatively low. The reason for this can be interpreted in several aspects. In PET-CT examination, 18F-FDG (a glucose analog) is taken into the cell with glucose transporter protein. Malignant cells have higher metabolic rates and express a greater number of specific membrane proteins than normal cells. As a result, 18F-FDG is taken up more by tumor cells. This is the basis of the FDG-PET imaging method. [16] As PET-CT is primarily a glucose measurement, it can give false positive results in cases where there is increased BM activity. Increased inflammatory markers, and inflammatory changes in the BM are examples of these conditions. Diffuse involvement may be associated with myeloid activation, which may also help to explain the low specificity of PET-CT in detecting BM involvement. [17] In addition, increased involvement in the sacral region may be observed in patients with B symptoms due to increased cytokine production. [2] Although the possibility is low, if PET/CT imaging were to be performed after BM biopsy, there could be a false increased involvement in the biopsy site.[18]

The limitations of this study are that it was performed retrospectively and with a limited number of patients. There is a need for further prospective studies with more patients.

Conclusion

The PET/CT method has high sensitivity in detecting the BM involvement of HL patients. Therefore, in patients with no BM involvement on PET-CT, there can be considered to be no need for the invasive and painful procedure of biopsy. However, since it may cause false positive staging due to low specificity, it will be more appropriate to perform BM biopsy in cases of high suspicion even though it is an invasive procedure.

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Original Article

Potential Novel Prognostic Factors in Malign Mesothelioma: Systemic Inflammatory Indices (SII) & Albumin-to-Globulin Ratio (AGR)

Malign Mezotelyomada Potansiyel Yeni Prognostik Faktörler: Sistemik İnflamatuar İndeksler (SII) ve Albümin-Globulin Oranı (AGO)

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ABSTRACT

Introduction: Malignant mesothelioma (MM) is rare with poor prognosis and often diagnosed at advanced stage. Systemic inflammatory indices (SII) may have prognostic value in cancer. Albumin is a negative acute phase reactant. We evaluated the prognostic significance of SII and albumin to globulin ratio (AGR) in MM followed-up at a single institute.

Methods: Fifty-six MM patients who met the inclusion criteria at our oncology cenrter were included in the study. Patients aged over 18 years with pathologically confirmed malignant pleural and peritoneal mesothelioma and no secondary malignancy followed up at our center were included in the study. Laboratory parameters for estimation of SII and AGR at diagnosis were obtained from database. Those with active infection, which might affect these parameters, those with a medical history of steroid use were excluded from the study.

Results: Median follow-up was 13.5 months. Most of the patients were female (58.9%). Median overall survival (OS) was 13 months. Median OS was 16 months in the pleural mesothelioma group and 9 months in the peritoneal mesothelioma group (p=0.982). Median OS was longer with lower platelet level, lower neutrophil to lymphocyte ratio (NLR) level and lower platelet to lymphocyte ratio (PLR) level (p1=0.001, p2=0.001 p3<0.001; respectively). On the other hand, median OS was longer with higher lymphocyte count, higher albumin level and higher AGR level (p1=0.032, p2=0.03, p3=0.003). Lymphocyte, Platelet count and AGR were determined as independent prognostic factors for OS according to multivariate cox regression analysis (p1=0.047, HR: 0.852; p2=0.011, HR: 2.502; p3=0.032, HR: 0.495, respectyively).

Discussion and Conclusion: It has been demonstrated that AGR, platelet and lymphocyte counts are independent prognostic factors for OS in MM.

Keywords: Malignant Mesothelioma, albumin, albumin-to-globulin ratio, systemic inflammatory indices (SII), NLR, PLR

ÖZET

Giriş ve Amaç: Malign mezotelyoma (MM) sıklıkla ileri evrede tanı alan kötü prognozlu nadir bir hastalıktır. Sistemik inflamatuar indeksler (SII) kanserde prognostik değerlere sahip olabilir. Albumin, negatif bir akut faz reaktanıdır. Sunulan calışmada MM takibinde SII ve albuminin globulin oranının (AGO) prognostik önemini değerlendirdik.

Yöntem ve Gereçler: Merkezimizde dahil edilme kriterlerini karşılayan 56 MM hastası calışmaya dahil edildi. 18 yaş üstü, patolojik olarak doğrulanmış malign plevral ve peritoneal mezotelyoma olan

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sekonder malignitesi olmayan hastalar calışmaya dahil edildi. Tanı anında SII ve AGR laboratuvar parametreleri veri tabanından retrospektif olarak kaydedildi. Bu parametreleri etkileyebilecek aktif enfeksiyonu olanlar, steroid kullaım öyküsü olanlar çalışma dışı bırakıldı.
Bulgular: Medyan takip süresi 13,5 aydı. Hastaların çoğunluğu (%58.9) kadındı. Medyan genel sağkalım (OS) 13 aydı. Median OS, plevral mezotelyoma grubunda 16 ay ve peritoneal mezotelyoma grubunda 9 aydı (p=0.982). Median OS, düşük trombosit seviyeleri, düşük nötrofil lenfosit oranı (NLR) seviyeleri ve düşük trombosit/lenfosit oranı (PLR) seviyelerinde daha uzundu (sırasıyla p1=0.001, p2=0.001 p3<0.001). Öte yandan, medyan OS yüksek lenfosit sayısı, daha yüksek albumin düzeyi ve daha yüksek AGR düzeyleriyle daha uzundu (p1=0.032, p2=0.03, p3=0.003). Lenfosit, trombosit sayısı ve AGR, multivariate cox regresyon analizine gore OS için bağımsız prognostik faktörler olarak belirlendi (p1=0.047, HR: 0.852; p2=0.011, HR: 2.502; p3=0.032, HR: 0.495, sırasıyla).
Tartışma ve Sonuç: Çalışmada AGO, trombosit ve lenfosit sayılarının MM'de OS için bağımsız prognostik faktörler olduğu gösterilmiştir.

Anahtar Kelimeler: Malign Mezotelyoma, albumin, albumin globulin oranı, sistemik inflamatuar indeksler, NLR, PLR

Introduction

Malignant mesothelioma (MM) is a rare neoplasm of serous membranes such as pleura, peritoneum, pericardium, and tunica albuginea [1]. It has poor prognosis with a median overall survival (OS) of around one year (range: 6-12 months) [2]. The incidence of pleural MM is approximately 10 to 30 fold higher than peritoneal MM [3]. The incidence is increasing worldwide, mainly due to occupational asbestos exposure [4]. There is a strong positive correlation between asbestos exposure and MM development at any localization. Respiratory exposure to asbestos has been reported as the main cause of pleural MM that accounts for approximately 70% of pleural MM cases who were documented for asbestos exposure [5].

Major histological subtypes are epithelioid, sarcomatoid, and biphasic (mixed) MM. Sarcomatoid MM has worse prognosis than epitheloid subtype [6]. 60% of MM patients present with stage III or IV disase at diagnosis [7]. In the literature, some factors including blood hemoglobin level and white blood cell count, Eastern Cooperative Oncology Group (ECOG) performance score and baseline symptoms have been reported to have prognostic significance [8,9]. However, their role as prognostic factors in MM are not so clear since most of clinical data are based on retrospective series in the literature because of its rarity and geographical distribution. Turkey, especially some regions such as Tuzköy and its nearby localizations tend to have relatively higher risk for MM because of erionit and others similar to asbestos structure in that region that may have role in development of MM [10]. Therefore, we should focus on MM in Turkey in terms of prognostic and predictive factors in MM.

In recent years, numerous studies, which have been conducted with inflammation-based markers, have obtained promising outcomes for revealing the prognosis in various cancers [11]. It has been demonstrated that systemic inflammation is associated with poor survival in many cancer types [12]. Inflammatory cells in the tumor microenvironment were shown to have significant effects on tumor development, and systemic inflammation blood markers may provide considerable information in predicting the prognosis [9]. Albumin and globulin are proteins that are the main component of serum. Albumin is a negative acute phase reactant which also reflects the nutritional status and systemic inflammatory response in cancer patients [13]. Globulin, the other main protein component of serum, has crucial roles in immunity and

inflammation [14]. Lower serum albumin level accompanied with higher globulin level may reflect inflammatory response in tumors. Recently, albumin to globulin ratio (AGR) has been reported to have prognostic value in various cancers [15]. However, its role has not been well studied in relatively rare tumors including MM. Therefore, we considered that AGR may have prognostic role in MM, besides other systemic inflammatory indeces such as neutrophil to lymphocyte ratio (NLR) and platelet to lymphocyte ratio (PLR).

Hence, this study was planned to determine the prognostic factors which impact OS, by assessing the retrospective data of patients with MM at our center, who had been followed up in a single center, in the light of the literature.

Material Methods

Upon retrospectively reviewing the data of 102 MM patients who were followed up in our cancer center between 2011 and 2020, 56 patients met the inclusion criteria for our study were included. Patients aged over 18 years with pathologically confirmed malignant pleural and peritoneal mesothelioma without any secondary malignancy were enrolled. Laboratory parameters for SII and AGR at diagnosis were obtained from the patients' database. Those with active infection or a medical history of steroid use, which might affect these parameters, were excluded from the study. The demographic data of the patients and their clinical characteristics were noted down from the patient files. During the study follow-up, disease-free survival (DFS) and progression-free survival (PFS) were calculated based on the recurrence in patients with early-stage and progression in patients with advanced stages. Moreover, OS was calculated by using the central record, according to the dates of the deaths (death notification form). NLR, PLR and AGR were calculated with the formula: Neutrophil count (/μL) / Lymphocyte count (/μL); Platelet count (10^9/L) / Lymphocyte count (/μL) and Albumin value (g/dl) / Globulin value (g/dl). The study was approved by the local ethics committe, ethical approvel number 2021-04/1125; approvel date:21/04/2021.

Statistical Analysis

Statistical analyzes were performed via the software of SPSS 25.0 (SPSS, Chicago, IL, USA). Mann-Whitney U test was used for comparison of nonparametric data, and Student T-test was used for comparison of parametric data. Chi-Square or Fisher's Exact test was used for comparison of categorical data. Optimum cut-off values that can be used to determine the prognostic significance of NLR, PLR, AGR, lymphocyte count and platelet count were determined by receiver operating characteristic (ROC) analysis. Kaplan–Meier method was used for survival analysis, and the Log-Rank test was used for the comparisons between groups. Prognostic factors affecting overall survival were determined by conducting multivariate analysis with the Cox proportional hazards model. Variables with a p value under 0.20 as a result of univariate analysis were evaluated in the cox-regression model. The results were considered statistically significant at $p<0.05$.

Results

Thirty-three (58.9%) of 56 patients in the study were female. Median age of the patients was 65 years (18-77). While 37 (66.1%) patients had pleural mesothelioma, 19 (33.9%) patients were diagnosed with peritoneal mesothelioma. The demographic and clinical characteristics of the patients are summarized in Table-1. At the time of diagnosis, 18 patients (32.1%) were operable, 34 patients (60.7%) were unresectable, and 4 patients (7.1%) were medically inoperable. Pathologically, 40 patients (71.4%) had epithelioid MM while 9 patients (16.1%) had

Table 1: Summary of Patient Characteristics

Characteristics		
Gender		
	Female	33 (58.9%)
	Male	23 (41.1%)
Median Age		65 (18-77)
Tumor Location		
	Plevra	37 (66.1%)
	Periton	19 (33.9%)
Asbest Exposure		
	No	16 (28.6%)
	Yes	16 (28.6%)
	Unknown	24 (42.9%)
Smoker		
	No	39 (69.6%)
	Yes	17 (30.4%)
ECOG PS		
	<2	33 (58.9%)
	≥2	23 (41.1%)
Symptoms at diagnosis		
	Localized pain	39 (69.6%)
	Dyspnea	32 (57.1%)
	Weight loss	11 (19.6)
	Fatigue	28 (50%)

ECOG PS: Eastern Cooperative Oncology Group Performance Status

biphasic MM and 7 patients (12.5%) had sarcomatoid MM subtypes. Grade 3-4 adverse effects related to chemotherapy occurred in 17 patients (30.4%). Five pleural MM patients (8.9%) received adjuvant radiotherapy. Pathological, surgical and medical treatment characteristics of the patients are summarized in Table-2.

Median follow-up period was 13.5 months. In the study, median OS was 13 months (95% CI =9.85-16.14). Median OS was 16 months (95% CI=10.04-21.95) in the pleural MM group while it was 9 months (95% CI = 6.38-11.61) in the peritoneal MM group and there was no significant difference for OS between these two groups (p=0.982). Median DFS of 18 patients who recurred after surgery was 12 months (95% CI=4.16-19.84). In 38 non-operated patients, median PFS was 7 months (95% CI=4.93-9.06) following first line treatment. Median PFS was 8 months (95% CI = 4.04-11.98) for pleural MM group, and it was 6 months (3.41-8.58) for peritoneal MM group, as well. (p=0.159) (Table-3).

Table 2: Baseline Characteristics of Surgery Pathology and Therapy

Characteristics	
Operation	
Operable	18 (32.1%)
Inoperable	34 (60.7%)
Medical Inoperable	4 (7.1%)
Type of Operation	
Pleurectomy/Decortication (Pleural)	8 (14.3%)
Debulking (Peritoneal)	10 (17.9%)
HIPEC	4 (7.1%)
Pathology	
Epithelioid	40 (71.4%)
Sarcomatous	9 (16.1%)
Biphasic	7 (12.5%)
IHC Staining	
Kalretinin	56 (100 %)
WT-1	43 (75.57%)
Cytokeratin 5/6	33 (58.9%)
Chemotherapy at Adjuvant in Operable Patients	
Platin + Pemetrexed	14 (77.8%)
Platin+Pemetrexed+Bevacizumab	1 (5.6%)
Not Received	3(16.7%)
Chemotherapy at First Line in Inoperable Patients	
Platin + Pemetrexed	23 (60.5%)
Platin+Pemetrexed+Bevacizumab	5 (13.2%)
Platin + Gemcitabine	1 (2.6%)
Not Received	3(7.9%)
Unknown	6(15.8%)
Chemotherapy at Relapse in Operable Patients	
Pemetrexed	2 (28.5%)
Vinorelbine	1 (14.2%)
Gemcitabine	3 (42.8%)
Not Received	1 (14.2%)
Chemotherapy at Progression in Inoperable Patients	
Gemcitabine	13 (46.4%)
Platin + Gemcitabine	1 (3.5%)
Pemetrexed	2 (7.14%)
Vinorelbine	1 (3.5%)
Not Received	11(39.2%)
Adjuvant Radiotherapy	5 (8.9%)

HIPEC: Hyperthermic Intraperitoneal Chemotherapy

Table-3: Survival Rates

	OS (month)
All patients (n:56)	13 (9.85-16.14)
Pleural	16 (10.04-21.95)
Peritoneal	9 (6.38-11.61)
	DFS (month)
Recurrence after surgery (n:18)	12 (4.16-19.84)
	First Line PFS (month)
Non operated patients (n:38)	7 (4.93-9.06)
pleural	8 (4.04-11.98)
peritoneal	6 (3.41-8.58)

The patients with better ECOG-PS at diagnosis had longer OS. Median OS was 22 months (95% CI=16.56-27-44) for the patients with an ECOG-PS <1 at the time of diagnosis whilst it was 7 months (95% CI=3.79-10.22) for the others with ECOG-PS >2 and the difference between these two groups was statistically significant (p=0.002). While median OS was 22 months (95% CI =7.53-36.46) in the operated patients, it was 11 months (95% CI=6.97-15.02) in the non-operated patients (p=0.014). Of the patients who had progression with first line treatment, 17 patients were followed-up with best supportive care (BSC) whereas 6 patients had second line chemotherapy. When these subgroups were compared for PFS, those who had second line chemotherapy had numerically longer PFS, however, the difference was not statistically different (6 months versus 3 months, p=0.141).

Optimum cut-off values for NLR, PLR, AGR, albumin, lymphocyte count, and platelet count which predicting OS were 3.0, 200, 1.07, 3.5, 1500, and 350, respectively. Median OS was 22 months (95% CI = 7.43-36.56) for the patients with lower NLR level while it was 9 months (95% CI = 4.00-13.99) for the patients with higher NLR level (p=0.001). Median OS was 26 months (95% CI = 11.66-40.33) for the patients with lower PLR level while it was 9 months (95% CI = 4.01-13.98) for the patients with higher PLR level (p<0.001). Median OS was 9 months (95% CI= 4.15-13.84) for the patients with lower AGR level while it was 22 months (95% CI =13.33-30.66) for the patients with higher AGR level (p=0.003). OS was significantly longer in the patients with higher albumin level (>3.5 g/dL). Median OS was 19 months (95% CI = 11.21-26.78) for the patients with an albumin value of >3.5g/dL, while it was 9 months (95% CI = 6.24-11.75) for the patients with an albumin value of ≤3.5 g/dL (p=0.03). OS was significantly longer in the patients with higher lymphocyte level (18 vs 10 months, respectively; p=0.032) while it was significantly shorter in the patients with higher platelet level (10 vs 28 months, respectively; p=0.001).

Multivariate cox regression analysis was performed with lymphocyte, AGR, albumin, NLR, PLR and Platelet. Lymphocyte, Platelet count and AGR were determined as independent prognostic factors for OS according to multivariate cox regression analysis [p=0.047, HR: 0.852 (95% CI= 0.674 –0.986) for lymphocyte count; p=0.011, HR: 2.502 (95% CI =1.233 – 5.076) for platelet count; p=0.032, HR: 0.495 (95% CI =0.260–0.942) for AGR, respectyively].

Discussion

In this study, in which 56 patients with malignant pleural and peritoneal MM were investigated retrospectively in a single cancer center, it was demonstrated that lymphocyte, AGR values and platelet count at the time of diagnosis are independent prognostic factors for OS. Median age of was 65 years, and when the literature was reviewed, it was found to be higher compared to other studies that have been conducted in Turkey [1,16].

Median OS was 13 months for all population. It increased to 16 months in the pleural MM subgroup and whereas it decreased to 9 months in the peritoneal MM group. It is well-

known that OS varies depending on the clinical characteristics of the patients with MM, such as stage at diagnosis, operability and pathological subgroups. In the series in which Dogan et al. examined patients with pleural and peritoneal MM, median OS was 22 months, while in a large series of 910 patients in which only patients with pleural MM were evaluated, median OS was determined to be 10 months [1,17]. Besides, in a study conducted on patients with peritoneal MM, median OS was determined as 11 months [18].

In the present study, the patients who were operated had a significant survival advantage when compared to those who were not operated (22 months vs. 11 months). It is well-documented that median OS in patients with pleural MM who underwent extrapleural pneumonectomy is 18 months and that a substantial number of patients achieve long-term survival [19]. In a study in which 27 patients with peritoneal MM were given intraperitoneal chemotherapy in addition to cytoreductive surgery, the 3-year survival was determined to be 67% [20]. Unlike this study, in presented study 10 out of 19 peritoneal MM patients were operated and only 4 received intraperitoneal chemotherapy.

It is well-established that the ECOG performance score is a prognostic factor in various cancers [21]. In a Taiwan study, which was conducted on patients with pleural MM, it was revealed that patients with an ECOG performance score of ≥2 had a poor prognosis [22]. Consistent with the literature, patients with low ECOG performance scores had a shorter OS in the presented study (22 months vs. 7 months). These differences in survival outcomes might be related to the heterogeneity of the studies, including the fact that some of them are retrospective, the number of patients, ECOG performance scores, pathological subtypes, operation status, whether intraperitoneal chemotherapy is given in peritoneal mesothelioma, and the difference in the chemotherapy protocols.

In the study, when the albumin levels > 3.5 and AGR >1.07 by using the ROC curve, the median OS was statistically significant at high albumin and AGR levels. Also, AGR was independent prognostic factors for OS. It is well-known that serum albumin level, which is simple, inexpensive, and widely available, is a negative acute phase reactant and decreases as inflammation increases [23]. Furthermore, as malnutrition is very common in cancer patients, serum albumin level is often used to assess malnutrition status [24]. It has been demonstrated in a study, which was conducted on various cancers, that serum albumin level is an independent predictive marker indicating malnutrition [25,26]. Total serum protein and albumin show the absorption, synthesis, and decomposition of body proteins. Moreover, albumin has anti-tumor activity and can reflect immune system functions into practice [27]. Globulin, which is the other major protein component of serum may rise in serum as a result of the accumulation of acute-phase proteins which are involved in inflammation [28]. Studies have found out that increased cytokines in cancers are associated with a rise in immunoglobulin. This situation corroborates the thesis that an elevated level of globulin may be associated with apoptosis inhibition and cancer progression [29]. Hence, the AGR derived from albumin and globulin could be used as a factor indicating cancer progression [28]. Considering these data, it was found out that increasing serum AGR before treatment in patients with malignant mesothelioma were associated with better survival, additionally AGR was also an independent prognostic factor for OS. Consistent with the presented study, pre-treatment low AGR was reported to be significantly associated with poorer OS, increased 5-year mortality rates besides higher relaps and progression rates in a meta-analysis of 15356 patients diagnosed with

various cancer types such as gastric cancer, colorectal cancer, breast cancer, larynx carcinoma, and hepatocellular cancer [15]. Moreover, the studies including many solid tumor types at different stages demonstrated that basal AGR at diagnosis was associated with a better OS, DFS and PFS [30,31,32].

It is well-documented that hypoalbuminemia is a poor prognostic factor in many cancers [33,34]. In the presented study, a significant OS difference was determined between the patients with a serum albumin level of >3.5g/dL and those with a level of ≤3.5 g/dL (22 months vs. 9 months). In paralel to this data, pre-treatment serum albumin level was defined as an independent prognostic factor for OS in pleural MM [25]. Consistent with the presented study, studies performed in the patients with peritoneal MM also revealed poorer overall survival as the serum albumin value decreased [18, 24].

In the study, when the cut-off values for NLR, PLR, platelet and lymphocyte were taken using the ROC curve, the median OS was statistically significantly lower at high NLR, PLR and platelet levels, while the median OS was statistically significant at high lymphocyte levels. Besides platelet and lymphocyte markers were independent predictive factors for prognosis. The efficacy of these blood inflammatory parameters has been reported in numerous studies conducted on patients with MM. In a meta-analysis of 1533 pleural MM patients, it was revealed that increased NLR was associated with poorer survival rates [9]. In another study, PLR was also determinned to have prognostic significance [35]. It is well-known that platelets have a crucial role in inflammation and have a prognostic significance [36]. In a meta-analysis, pre-treatment high platelet count was shown to be associated with a poorer OS [37]. Lymphocytes act as tumor suppressors by inducing cytotoxic cell death and inhibiting tumor cell proliferation and migration [38]. Tumor-infiltrating lymphocytes can activate an effective antitumor cellular immune response [39]. Thus, as demonstrated in the presented study, increased lymphocyte counts may be associated with better survival outcomes.

This study has some limitations. It was retrospective, and a prospective multicenter study would be much better in terms of evaluating the prognostic factors of malignant mesothelioma. In this study, there is a risk of bias in some results due to the lower number of patients and missing data.

Conclusions

In this study, it has been demonstrated that AGR, platelet and lymphocyte counts are independent prognostic factors in MM. Higher albumin levels and AGR are associated with better survival. Large prospective clinical trials will provide better information and could reduce the possibility of bias.

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Case Report

MRI Findings of Infundibular Craniopharyngioma: Two Case Reports

İnfundibular Kraniofaringiomanın MR Görüntüleme Bulguları: İki Olgu Sunumu

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ABSTRACT

Primary infundibular craniopharyngioma is a relatively unusual disease due to its location, which usually results in late diagnosis. Two female patients are referred to the radiology clinics because of bitemporal hemianopsia detected in visual assessment at different times.

Infundibular lesions were detected with MRI both patients and craniopharyngioma was considered as the primary diagnosis. Both masses are operated and the diagnosis of craniopharyngioma is proven pathologically.

Keywords: MRI, infundibular Craniopharyngioma, Pituitary infindubulum

ÖZET

Primer infundibular kraniofarenjiyoma, konumu nedeniyle nispeten nadir görülen ve genellikle geç tanı alan bir hastalıktır. Farklı zamanlarda norolojik muayenelerinde bitemporal hemianopsi tespit edilen iki hasta MRG incelemesi için radyoloji kliniğine refere edildi. Her iki hastada da MRG ile infindubüler yerleşimli lezyonlar tespit edildi ve kraniofaranjiyom birincil tanı olarak düşünüldü. Her iki kitle de ameliyat edildi ve kraniofaranjiyom tanısı patolojik olarak doğrulandı.

Anahtar Kelimeler: MRG, İnfundibular Kraniofaringioma, Hipofizer İnfundibulum

Introduction

Craniopharyngiomas account for 1–4% of all intracranial tumours and 20% of the tumours of the sellar and chiasmatic region [1]. The tumor has two age peaks, the one occuring in children and the other one in adults between the 4th and 6th decades despite it's more common in childhood than adulthood period. Craniopharyngiomas arise from remnants of embryonic canal extending from the oropharynx to the median eminence and infundibulum. According to this theory, any place along this canal may serve as a site of tumor origin [2]. Although the epicenter of the lesions is usually suprasellar (90%), sellar or infrasellar region; anterior, middle and posterior cranial fossas, retroclival region, sphenoid bone, nasopharynx, cerebello-pontine angle and even pineal gland are other rare sites of the tumor development [3]. Infundibulum along the embryonic canal is another potential site for tumor origin. However, primary infundibular cranio-pharyngioma is a rare disease and uncommonly early diagnosed until it grows towards the suprasellar or parasellar regions. It usually presents with neurological complications like headache, visual disturbance and symptoms related with hypothalamic or hypophyseal gland dysfunction [4].

Here we report unusual infundibular cranio-

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pharyngioma cases which is pathologically confirmed and primarily arising from the infundibulum. We want to discuss imaging findings and differential diagnosis of infundibular craniopharyngioma.

Case Report

Case 1: A 32-year-old female patient has been admitted to the neurology outpatient clinic with complaints of persistent vision problems and headache for one month. Bitemporal hemianopsia is detected in the visual examination.

CT showed a hypodens mass with punctate peripheral calcifications in the suprasellar area. MRI revealed that the mass was originating from the pituitary infindubulum and containing cystic and solid areas, approximately 20x20 mm in size. The lesion compresses the optic chiasm posteriorly and is closely adjacent to vascular structures. The pituitary gland had a normal appearance. Based on these findings, radiological diagnosis was considered to be infindubular craniopharangioma (Figure 1a-c). Infundibular craniopharyngioma was confirmed histo-pathologically. On the 2nd year follow-up MRI, there was a mass lesion measuring 15 x 10 mm consistent with residual or recurrent tumor(Figure 1d).

Case 2: A 61-year-old woman suffering from visual disturbance for 2 months admitted to the hospital. Visual examination of the patient revealed bitemporal hemianopsia. An infundibular mass was demonstrated as intermediate signal intensity on T1-weighted MR images and high signal intensity similar to CSF corresponding to cystic changes on T2-weighted images. It showed peripheral and nodular enhancement pattern on postcontrast T1-weighted images. No restriction of diffusion is noted on diffusion weighted images. The tumor was extending from hypophyseal infundibulum to hypothalamus and compressing optic chiasm. (Figure 2 a-c) The initial radiological diagnosis of the lesion was craniopharyngioma, and then the lesion was decided to be operated on. Histopathological results confirmed infundibular craniopharyngioma. Follow-up MRI images two months after the operation showed no recurrence or residual tumor.

Discussion

Craniopharyngiomas are histologically benign tumors orginating from squamous epithelial cells of Rahtke's cleft. They usually involve sellar-suprasellar region and invade or extend to clinically important structures as optic chiasm and hypothalamus. It is more common in childhood than adulthood period. However our two patients were adult. Although craniopharyngiomas usually arise from Rathke's cleft, they can also arise from embryonic cells located anywhere along the craniopharyngeal canal. [5].

Infundibular involvement may be seen in many conditions as well as in craniopharyngiomas. It is important to make a differential diagnosis of infundibular diseases in order to treat appropriately. Infundibular lesions are generally classified into three categories as neoplastic, inflammatory-infectious and congenital-developmental. Neoplastic lesions include astrocytoma, ependymoma, germinoma, pleomorphic xanthoastrocytoma, lymphoma, prolactinoma, metastasis and craniopharyngioma. The first diagnosis to be considered in the absence of primary tumor in isolated stalk masses is craniopharyngioma.The size of infundibulum usually should be lesser than basilar artery at clivus level. More than this size might be a remarkable point for infundibular lesions. In many cases, hypothalamus is involved together with the infundibulum. The lesions involving both pituitary stalk and hypothalamus may result in diabetes insipidus.

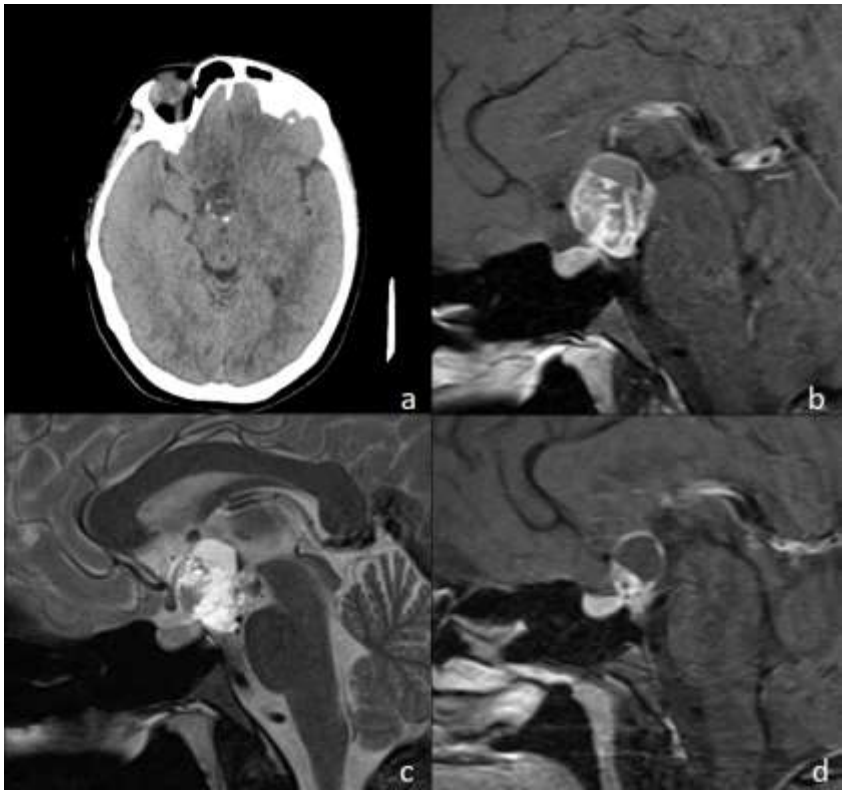

Figure 1(a-d):Non-contrast CT shows a mass with peripheral punctate calcifications in the suprasellar area. (a).Solid lesion of stalk with cystic-necrotic component is shown on contrast enhanced T1-weighted sagittal image(b) and T2-weighted sagittal image(c).The mass copresses the optic chiasm from the posterior and causes visual symptoms(c).The remaining mass is demonstrated in the postop contrast-enhanced MR image taken two years after surgery(d).

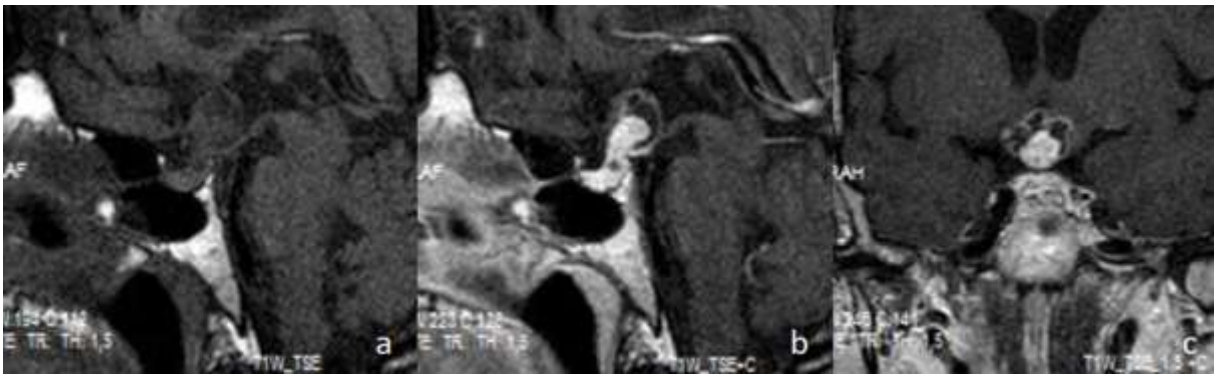

Figure 2(a-c). Sagittal non-contrast T1-weighted MRI shows heterogeneous suprasellar mass lesion (a). Contrast-enhanced solid lesion of stalk with superior cystic-necrotic component demonstrates on contrast enhanced T1-weighted sagittal (b) and coronal (c) images.

Pituitary adenomas may cause other endocrinologic problems due to excessive secretion of hormones. Germinomas which usually present as a tumor of pineal gland or hypothalamus, can also be seen in primarily infundibulum [6]. Metastasis to infundibulum are usually from breast and lung cancers. In the presence of primary tumor, metastasis can be considered primarily, but neither of our patients had primary tumors. Leukaemia, lymphoma and atypical/malignant meningiomas which are aggressive tumors of

hypothalamic-suprasellar region are other rare tumors can involve infundibulum [1]. Ratke's cleft cysts should be taken into accont if there is a cystic tumor of infundibulum. All of these tumors are in the differential diagnosis of infundibular craniopharyngiomas. However, there are some granulomatous diseases of the infundibulum that may resemble neoplasms. Sarcoidosis, tubercu-losis, langerhans cell histiocytosis are such kind of granulomatous diseases that involve infundibular stalk. Therefore, MRI is an important imaging method in the differential diagnosis of infundibular lesions.

In our cases, infundibular involvement is due to a true neoplastic pathology called infundibular craniopharyngioma. Visual impairment is one of the major and earliest presenting symptoms of patients harboring craniopharyngiomas and also a potential complication of the surgical treatment [7]. The first symptom in our patients was visual problems.Also it is usually diagnosed late unless complains of headache, polyuria and polydipsia or complains due to compression of optic chiasm consist. After diagnosis of craniopharyngioma, the next step is to choose appropriate treatment modality. There is a therapeutic dilemma for craniopharyngioma, so the approach is important when deciding on conservative or aggressive treatment. Conservative treatment may be an alternative management for early diagnosed small tumors especially for the tumors which are not extending to optic chiasm. However, there is no consensus on treatment options of larger lesions extending to optic chiasm. Complete surgical resection is usually indicated because it is believed that craniopharyngiomas are curable tumors. It is important to conserve infundibulum but it is sometimes sacrificed for total resection. Another alternative treatment modality for some authors is limited surgery followed by radiotherapy [8,9]. Surgical treatment was preferred in both of our patients. While total resection was achieved in our second case, our first case is being followed due to a residual mass.

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