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Contents ▼

Lead aVR in Electrocardiography: Clinical Usefulness

Elektrokardiyografide aVR Derivasyonu: Klinik Yararlılığı

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ABSTRACT

Although the electrocardiogram (ECG) contains 12 leads, lead aVR is frequently neglected and the interpretation is made from the other 11 leads. However, lead aVR may contain valuable information and can influence the decision in many clinical situations. Among these situations, ischemic heart disease and rhythm-conduction disturbances are the leading ones but not limited to these abnormalities. In this paper, various situations in which lead aVR can significantly contribute to the interpretation of the ECG are reviewed.

Keywords: Electrocardiography, aVR, lead

ÖZ

Her ne kadar elektrokardiyogram (EKG) 12 derivasyondan oluşsa da aVR derivasyonu sıklıkla ihmal edilmekte ve yorumlama diğer 11 derivasyon üzerinden yapılmaktadır. Bununla birlikte, aVR derivasyonu da değerli bilgiler içerebilir ve birçok klinik durumda tanıya etki edebilir. Bu durumlar arasında iskemik kalp hastalıkları ve ritim-iletim bozuklukları başta gelmektedir, ancak bu durumlarla sınırlı değildir. Bu yazıda, aVR'nin EKG yorumlanmasına önemli katkıda bulunabileceği çeşitli durumlar gözden geçirilmiştir.

Anahtar Kelimeler: Elektrokardiyografi, aVR, derivasyon

Introduction

Since the first recording of the electrical activity of the heart by Einthoven, the electrocardiogram (ECG) has been an essential diagnostic tool in cardiology (1). After the addition of augmented leads by Goldberger, the ECG has been standardized as a 12 lead-ECG with recording from 10 different sites (2). However, in a study by Pahlm et al. (3) lead aVR was reversed to its mirror image and interpreters were asked to interpret the 12-lead surface ECG. The results showed that 80-94% of interpreters had not detected when lead aVR was reversed (3). It was apparent that most of the interpreters were neglecting lead aVR. However, lead aVR may provide important information about the heart. In this review, some of the instances where lead aVR may play an important role in the diagnosis are presented under the subtitles of ischemic heart disease, rhythm-conduction abnormalities, and miscellaneous conditions (Table 1).

Ischemic Heart Disease

In acute myocardial infarction, lead aVR may help to predict the occlusion site of culprit coronary artery. ST segment elevation or depression or R wave slurring in lead aVR may have some implications. It has been suggested that in acute inferior myocardial infarction, the absence of >1 mm ST depression in lead aVR is 93% specific for root cause analysis occlusion as the culprit artery (4). If the ST depression is ≥1 mm, the culprit artery is left circumflex artery with a specificity of 80% and sensitivity of 96%. However, this needs to be further evaluated because of contradicting data. Baptista et al. (5) have reported that ST depression in lead aVR is not superior to classical criteria, which were accepted as ST depression in lead DI; ST depression in

leads V1 and V2, ST elevation in lead DIII > DII, ST depression in V3/ST elevation in DIII ratio >1.2. Different definition of the infarct related artery, which was defined as the artery with the most severe lesion, may explain the difference. Another method for evaluating ST segment changes in lead aVR is comparison with lead V1. The simultaneous impairment of blood flow in both the left circumflex and the left anterior descending artery could make the ST-segment vector more perpendicular to V1, resulting in less elevation of ST-segment in lead V1. In accordance with this hypothesis, Yamaji et al. (6) have found that greater ST-elevation in aVR than in V1 is 81% sensitive and 80% specific for left main coronary artery disease (4,7). Another explanation for the ST elevation in lead aVR could be the involvement of the basal septum by acute MI. The lead aVR faces the basal septum more than any other lead. Therefore, the involvement of basal septum, which has dual perfusion from both the right coronary artery and the left anterior descending artery, may result in ST elevation in lead aVR.

The perfusion from the left anterior descending artery is mediated through the first septal branch. The occlusion of the first septal branch or the left anterior descending artery proximal to the first septal branch may also cause ST elevation in lead aVR. This hypothesis was confirmed by Aygül et al. (8) and Engelen et al. (9), who found that ST elevation in aVR in acute myocardial infarction was predictive of left anterior descending artery occlusion proximal to the first septal perforator branch. However, due to dual perfusion of basal septum, ST elevation in aVR in acute anterior myocardial infarction should suggest multivessel disease or left main coronary artery disease that prevents collateral perfusion. In fact, the relation between ST

elevation in aVR and multivessel or left main coronary disease has been reported (10,11,12,13). Kosuge et al. (14) have also studied the utilization of lead aVR in predicting patients who are candidate for surgery of angioplasty. This discrimination is important since clopidogrel loading is beneficial in patients who will undergo angioplasty while it may be harmful for patients who will be surgically treated.

Another hypothesis about the ST elevation in lead aVR is that lead aVR is the reversal of leads V5 and V6, and therefore, any situation that causes ST depression in V5 and V6 could cause ST elevation in lead aVR. An example is the anterolateral subendocardial ischemia, which may cause reciprocal ST elevation in lead aVR as well as ST depression in leads V5 and V6 (15,16). According to this hypothesis, occlusion of the first diagonal branch may also cause ST elevation in aVR (17). There is also the possibility that the ST elevation in lead aVR in the study of Sclarovsky et al. (18) might have resulted from occlusion of first diagonal branch by emboli coming from the thrombus in the left main coronary artery. Also, the study by Martínez-Dolz et al. (19) demonstrated that ≥0.5 mm ST elevation in aVR is 90% specific for predicting the involvement of first diagonal branch or first septal perforator branch (including occlusions proximal to these branches). However, it was also noted that sensitivity was only 29%. The common feature of all these studies of acute anterior myocardial infarctions is that the ST elevation in lead aVR has high specificity but low sensitivity for predicting the occlusion site.

Recognition of the extension of an acute myocardial infarction is important in the prognosis of the disease. The lead aVR may also be helpful in estimating the infarct area. The lead -aVR is frequently used for this purpose. In conventional ECG, precordial leads V1 to V6 show an orderly progression in waveform morphology. On the other hand, there is a gap in the sequential order of frontal leads. Although there is 30º angle between lead aVL and I and between leads II and aVF or leads aVF and III, the angle between lead I and II is 60º and angle between leads III and aVR is 90º. The mirror image of lead aVR may alleviate these gaps because it is 30º apart from both lead II and I. Besides, the use of lead -aVR may enable the interpreters to evaluate the frontal leads in a sequential order; aVL, lead I, -aVR, lead II, aVF and lead III. From this point of view, ST elevation in -aVR may show lateral involvement in inferior myocardial infarctions and inferior involvement in lateral myocardial infarctions (20).

The degree of ST-segment depression is also important. In patients with anterolateral myocardial infarction, ≥0.5 mm ST-segment depression in aVR is an independent predictor of predischarge left ventricular ejection fraction <35% despite successful reperfusion (21). In such patients, the need for intraaortic balloon pump and percentage of TIMI flow grade

Table 1. Areas where lead aVR can be helpful
A. Ischemic heart disease
a. Prediction of culprit artery in acute myocardial infarction b. Estimating the extension of acute myocardial infarction c. Assessment of prognosis in acute myocardial infarction d. Evaluation of exercise electrocardiography
B. Rhythm and conduction abnormalities
a. Diagnosis of left anterior hemiblock, complete or incomplete right bundle branch block b. Prediction of origin of ventricular arrhythmia c. Prediction of origin of supraventricular arrhythmia d. Prediction of arrhythmia in hypertrophic cardiomyopathy or intoxication of tricyclic antidepressants
C. Miscellaneous conditions
a. Diagnosis of electrode misplacement b. Rotation of heart c. Right ventricular hypertrophy d. Acute pericarditis e. Hypertrophic cardiomyopathy f. Postpartum cardiomyopathy g. Acute pulmonary embolism

0-1 are more frequent. If the depression in aVR exceeds 1 mm in patients with inferior myocardial infarction, myocardial reperfusion is 8.41 times less likely to have occurred (22). The slurring of R wave in lead aVR is 70% sensitive and 93% specific for right ventricular involvement in acute posterior wall myocardial infarction (23).

In an analysis of The Tampere Acute Coronary syndrome (24) study, a positive T-wave in lead aVR was associated with higher 10-year mortality. The authors argued that positive T-wave in aVR could represent an unfavorable post myocardial infarction remodeling.

Lead aVR can also be used in the interpretation of exercise ECG. It has been reported that ST elevation in aVR accompanying ST depression in V5 is 50% sensitive and 92% specific for predicting left main or proximal left anterior descending artery

Figure 1. The exercise electrocardiography (ECG) of a patient. Panel A: Baseline ECG. Panel B: ECG at peak exercise. Note that there is an ST elevation in aVR despite minimal change in V1. The coronary angiography of this patient revealed a significant lesion at left main-left anterior descending-left circumflex artery bifurcation. There were also significant lesions on the right coronary artery (From courtesy of Dr. Naip Tuna)

stenosis or multivessel disease (25) (Figure 1). ST changes during the recovery phase are also important. Inclusion of aVR to interpretation can increase the accuracy of the test (26,27). Furthermore, inversion of positive T-waves in aVR during exercise test suggests myocardial viability in patients with Q-wave myocardial infarction (28).

Rhythm and Conduction Abnormalities

Potential use of aVR in the assessment of rhythm and conduction abnormalities is usually complementary. In left anterior hemiblock, the presence of terminal R wave in aVR and the terminal R wave peak in aVR occurring later than that in aVL is diagnostic (29). In the diagnosis of right bundle branch block, lead aVR is not usually used but the presence of QR or rSRᴵ pattern in aVR is supportive.

The importance of aVR has increased after the introduction of electrophysiology to routine practice. During electrophysiological studies of ventricular tachycardia, accurate prediction of the origin of the premature beats is extremely important for reducing radiation time. It is frequently difficult to differentiate the origin of tachycardia between right ventricular outflow tract and pulmonary artery. In tachycardia originating from pulmonary artery, the ratio of the amplitude of Q-wave in aVR to the amplitude of Q-wave in aVL is increased (Figure 2) (30). However, this finding is also present in ventricular tachycardia originating from posterolateral portion of the right ventricular outflow tract (31). The QRS wave duration in lead aVR may be an important predictor for successful ablation of ventricular tachycardia or ventricular premature beats originating from right ventricular outflow tract. The duration of QRS wave in lead aVR was found to be inversely related to the success of ablation (32).

The QRS deflection may help delineate the origin of ventricular premature depolarizations. Barmeda et al. (33) have found in a study with small sample size that the origin of the ventricular premature depolarization is more likely to be basal septal or basal left ventricle if QRS deflection in aVR is opposite with aVL, while it is more likely to be other sites of the left ventricle if they are in the same direction.

The lead aVR may also help in differential diagnosis of narrow complex tachycardia. The ST elevation in aVR is seen in 71% of atrioventricular reentrant tachycardia, 31% of atrioventricular nodal reentrant tachycardia and 16% of atrial tachycardia (34). Therefore, ST elevation in aVR suggests that it is a reentrant tachycardia involving the atrioventricular node. In a study by Kuo et al. (35), it was shown that p wave polarity was negative in all patients with paroxysmal atrial tachycardia originating from the superior vena cava or right upper pulmonary vein. The distinction between these two is possible after the assessment of V1 and aVL. The polarity of
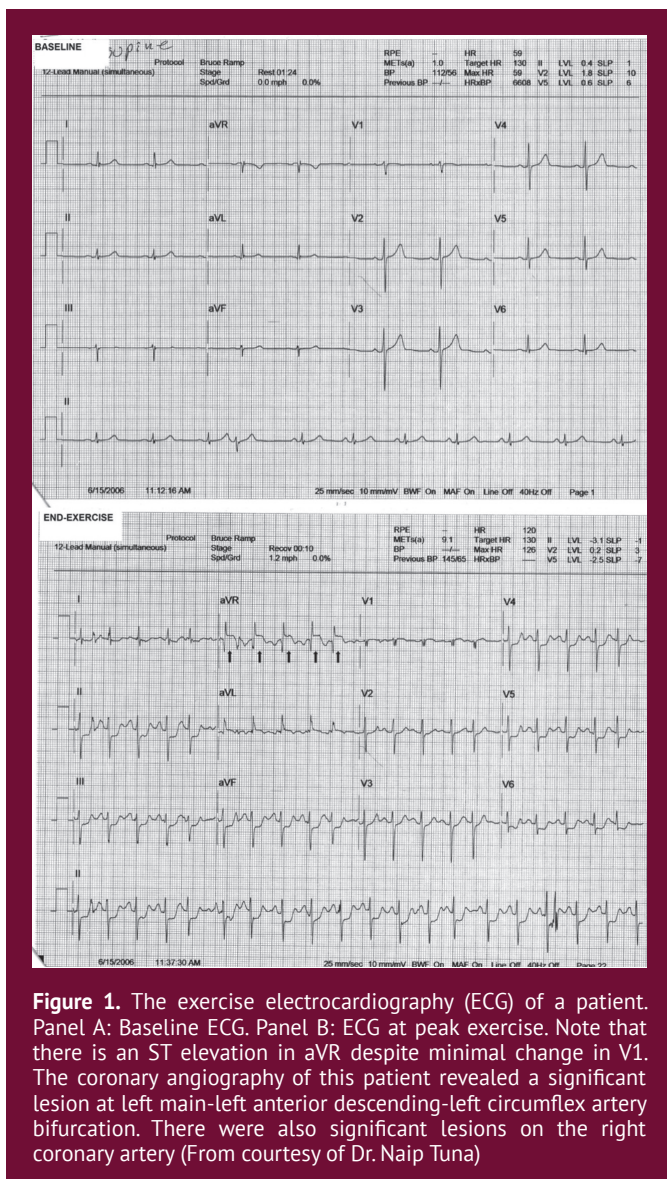
p-wave in aVR may also be helpful in locating the origin of right atrial tachycardia. The negative polarity of p-wave is 100% sensitive and 93% specific in identifying tachycardia originating from crista terminalis in patients with right atrial tachycardia (36). The lead aVR may also be used in differentiating upper loop reentry from typical atrial flutter. The amplitude of flutter wave is significantly lower in upper loop reentry than in typical atrial flutter (37).

The configuration of QRS in aVR may also help in predicting future cardiac arrhythmias. There are at least two types of patients for whom this might be helpful: patients with hypertrophic cardiomyopathy and patients with tricyclic antidepressant intoxication. In patients with hypertrophic cardiomyopathy, positive QRS wave in aVR is a predictor for inducible ventricular tachycardia (38). These patients are at greater risk for future sudden death. Arrhythmia is also an important problem for patients with tricyclic antidepressant intoxication. The most powerful electrocardiographic predictor of future arrhythmia and seizure is suggested to be R wave amplitude ≥3 mm in aVR (39). Buckley et al. (40) compared various criteria and found that the criterion of R/S >0.7 in aVR is the most powerful predictor. However, its low negative predictive value (41%) must be compensated by using other criteria.

Miscellaneous Conditions

The misplacement of electrocardiographic leads is a major problem for the interpreters of the ECG. If not recognized, it may result in erroneous diagnosis and inappropriate management. Ho and Ho (41) have developed an algorithm to uncover the misplacement of electrocardiographic leads not involving the right leg lead. According to this algorithm, the evaluation begins with the localization of the true aVR. In this algorithm, aVR may show the configuration of aVF or aVL. The similarity of QRS configuration to any other limb lead must prompt the interpreter to take lead misplacement into consideration (Figure 3).

The displacement of the heart itself is also a frequent problem in interpretation. The QR pattern in aVR should suggest the prominent clockwise rotation of the heart, while the QS or rS pattern is suggestive of intermediate clockwise rotation.

In the diagnosis of right ventricular hypertrophy, aVR is not routinely used because of its low specificity. However, when the other findings are not conclusive, aVR may provide supportive data. The QR pattern in aVR is found in type A right ventricular hypertrophy. It should be kept in mind that this finding is also found in clockwise rotation of the heart, backward displacement of apex, incomplete or complete right bundle branch block and inferolateral myocardial infarctions.

Acute pericarditis is characterized by ST elevation in all leads except for aVR, which usually reflects reciprocal changes. The electrocardiographical signs of acute pericarditis change according to the stage. In all stages, the aVR shows opposite changes, i.e. ST segment depression, T wave negativity and positivity and PR segment elevation (42,43).
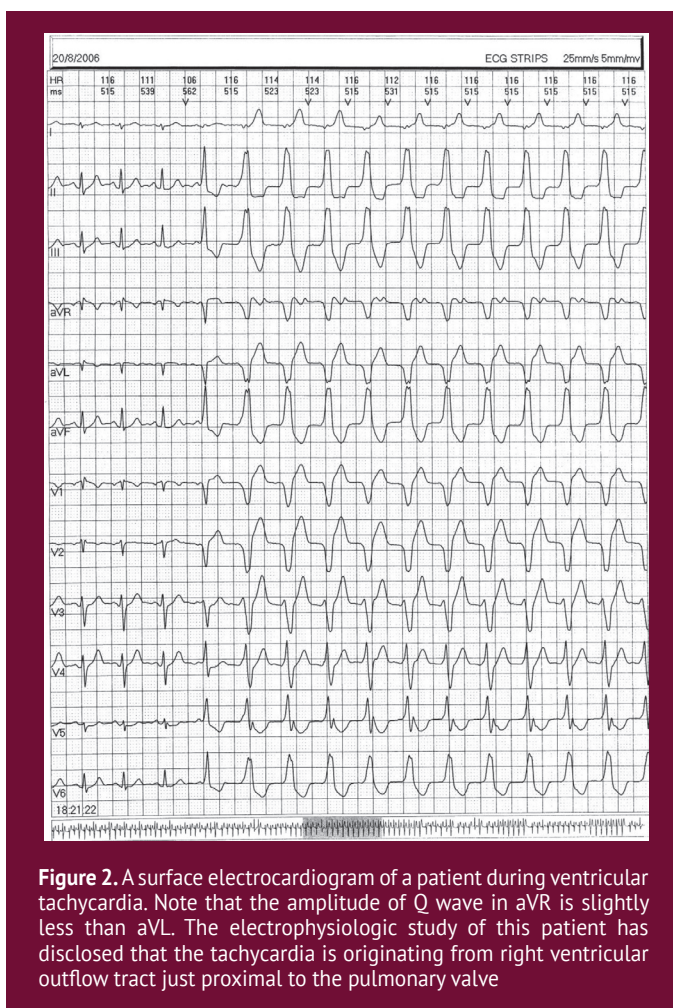
Figure 2. A surface electrocardiogram of a patient during ventricular tachycardia. Note that the amplitude of Q wave in aVR is slightly less than aVL. The electrophysiologic study of this patient has disclosed that the tachycardia is originating from right ventricular outflow tract just proximal to the pulmonary valve
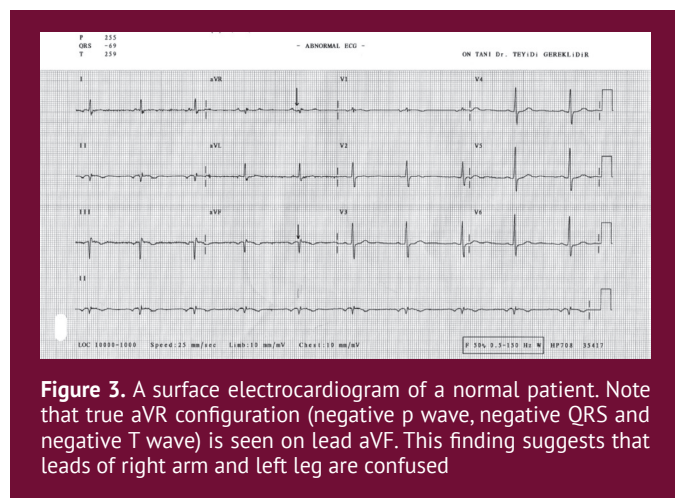
Figure 3. A surface electrocardiogram of a normal patient. Note that true aVR configuration (negative p wave, negative QRS and negative T wave) is seen on lead aVF. This finding suggests that leads of right arm and left leg are confused

Hypertrophic cardiomyopathy is usually characterized by left ventricular hypertrophy. Right ventricular involvement is very rare. In a few cases of the involvement of anterobasal wall of right ventricle, R wave amplitude in aVR was noted to be increased (44).

Ekizler et al. (45) has investigated the prognostic role of lead aVR in postpartum cardiomyopathies in 82 patients. They have found that T-wave amplitude in lead aVR predicted primary endpoint, which was defined as was defined as cardiac death, arrhythmic events, or persistent left ventricular systolic dysfunction, with a sensitivity of 100% and specificity of 100%.

In a study by Yelgeç on acute pulmonary embolism patients, mortality was higher in patients presenting with positive T-wave in lead aVR. This finding was also related to lower systolic blood pressure, which means that the disease was more severe in such patients.

Highlights

• Lead aVR is important in a wide variety of cardiac disease.
• In interpreting lead aVR, QRS deflection, ST segment elevation and direction of T-wave are important.
• Changes in lead aVR in ischemic heart diseases are usually indicative of more severity and worse prognosis.

Conclusion

The 12-lead ECG must be interpreted as a whole. No lead should be overlooked during interpretation. Lead aVR must be included in the analysis of the ECG. It has special importance in various cardiac conditions, including acute myocardial infarction, arrhythmias, conduction defects and electrode misplacements.

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The Importance of Single or Combined Use of Measurement Uncertainty and the Reference Change Value in the Diagnostic Evaluation of Biochemical Tests

Biyokimyasal Testlerin Tanısal Değerlendirmesinde Ölçüm Belirsizliği ve Referans Değişim Değerinin Tek Başına veya Birlikte Kullanımının Önemi

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ABSTRACT

Background: We aimed to improve the accuracy and reliability of our test results by calculating the measurement uncertainty (MU) and reference change value (RCV) for routine biochemical parameters.

Materials and Methods: For the MU estimation, 23 different routine biochemistry parameters were included in the study. For the RCV calculation of the tests, Fraser and logarithmic transformation formulas were used. The MUs were compared to the total allowable error recommendations defined by the CLIA and Westgard.

Results: When the estimated MU results of our laboratory were compared with the international limits, it was found that albumin, amylase, alanine transaminase, total bilirubin, direct bilirubin, blood urea nitrogen, calcium, creatinine kinase, chlorine, creatinine, glucose, potassium, lactate dehydrogenase, lipase, magnesium, sodium, total protein, phosphorus, C-reactive protein (CRP), aspartate transaminase, β-human chorionic gonadotropin, creatine kinase (CK)-MB (mass) and troponin-I test results were compatible with CLIA'88 limits, but the MU results of albumin, calcium, chlorine, magnesium, sodium and total protein were not compatible with Westgard limits. The RCV results, using Fraser approach, of CRP, CK, direct bilirubin, total bilirubin, lipase and troponin I-tests showed a wide range because of the high biological variations. The RCV's calculated limit value for CRP in the decreasing direction exceeded 100%, which makes it impossible to use. However, when RCV was recalculated with logarithmic conversion formula, more usable results were obtained.

Conclusion: Each laboratory should calculate MU values to bring the reliability of test results close to international limits. Logarithmic transformation formulas should be used in the RCVs calculation of tests with high biological variation, such as CRP. In addition, MU and RCV should be given with the test results to improve diagnostic accuracy.

Keywords: Measurement uncertainty, reference change value, biological variation, logarithmic transformation

ÖZ

Amaç: Rutin biyokimyasal parametreler için ölçüm belirsizliğini (ÖB) ve referans değişim değerini (RDD) hesaplayarak test sonuçlarımızın doğruluğunu ve güvenilirliğini artırmayı amaçladık.

Gereç ve Yöntemler: ÖB tahmini için 23 farklı rutin biyokimya parametresi çalışmaya dahil edildi. Testlerin RDD hesaplaması için Fraser ve logaritmik dönüşüm formülleri kullanıldı. ÖB'leri, CLIA ve Westgard tarafından tanımlanan ve izin verilen toplam hata limitleriyle karşılaştırıldı.

Bulgular: Laboratuvarımızda elde edilen ÖB sonuçları uluslararası limitlerle karşılaştırıldığında, albümin, amilaz, alanin aminotransferaz, total bilirubin, direkt bilirubin, kan üre azotu, kalsiyum, kreatinin kinaz, klor, kreatinin, glukoz, potasyum, laktat dehidrogenaz, lipaz, magnezyum, sodyum, total protein, fosfor, C-reaktif protein (CRP), alanin transaminaz, β-insan koryonik gonadotropini, kreatin kinaz (CK)-MB (mass) ve troponin-I testlerine ait ÖB sonuçlarının CLIA'88 limitlerini karşıladığı, ancak Westgard limitlerine göre albümin, kalsiyum, klor, magnezyum, sodyum ve total protein sonuçlarının başarısız olduğu saptandı. Biyolojik varyasyonlarının yüksekliği nedeni ile CRP, CK, direkt bilirubin, total bilirubin, lipaz ve troponin I-testlerinin klasik Fraser yaklaşımı ile hesaplanan RDD sonuçları, geniş aralık göstermekteydi. CRP için hesaplanan RDD'nin azalan yöndeki sınırı, kullanımı mümkün olmayacak şekilde yüzde 100'ü aşmıştı. Oysa RDD, logaritmik dönüşüm formülü ile tekrar hesaplandığında daha kullanılabilir sonuçlara ulaşıldı.

Sonuç: Her laboratuvar, test sonuçlarının güvenilirliğini uluslararası sınırlara yakınlaştırmak için ÖB değerlerini hesaplamalıdır. CRP gibi biyolojik varyasyonu yüksek olan testlerin RDD hesaplamasında logaritmik dönüşüm formülleri kullanılmalıdır. Ayrıca, ÖB ve RDD'nin tanısal doğruluğunu artırmak için test sonuçları ile birlikte verilmelidir.

Anahtar Kelimeler: Ölçüm belirsizliği, referans değişim değeri, biyolojik varyasyon, logaritmik transformasyon

Introduction

Nowadays, more accurate, reliable and reproducible results can be achieved with quality improvement programs and improvements in clinical laboratories in order to be sufficiently beneficial to patients. However, despite all these improvements, results are not completely accurate or reliable. Medical decisions contain some margin of error since they are affected by laboratory results. Therefore, when reporting the result of a measurement, a numerical indicator (expressing the quality of the result) containing this doubt should be added. Without such an indication, the results could not be compared with each other or with the values given in the standards (1,2). This can only be achieved by calculating the uncertainty of values obtained from the measurement and by reporting it with results.

The Guide to the expression of uncertainty in measurement (GUM) defines measurement uncertainty as "a parameter indicating the distribution of probabilities reported along with the measurement result and attributable to the measurement result". The International Vocabulary of Metrology - Basic and General Concepts and Associated Terms (VIM, item 2.26), on the other hand, defines it as a non-negative parameter that characterizes the distribution of values attributed to the measured (2). GUM and the Eurachem guideline adapted to the GUM in chemical measurements calculate uncertainty according to the bottom-up approach. This approach is based on identifying each potential source of uncertainty and calculating the individual uncertainty values for each component. The top-down approach is a method that uses existing laboratory test performance information (method validation, intra- and inter-laboratory CV data) (3).

The purpose of the measurement uncertainty is to help evaluating whether the result of one sample is significantly different from the other results. However, the uncertainty of measurement is not sufficient by oneself for the significance of the difference between the results obtained in successive measurements. To solve this issue, different parameters or concepts should be implemented (4,5). One of the most applicable parameters is the reference exchange value (RCV), which tests the significance between two measurements by taking analytical and biological variations into account.

There are two components of biological variation, intra-individual and inter-individual. Intra-individual biological variation (CVI) is expressed as random fluctuations of components in the human body at the homeostatic set points. Random fluctuations, caused from individual changes, develop depending on the aging process, sex, weight, diet, exercise, hemostasis, daily or seasonal rhythms and, of course, pathological status and treatment. The variation in these differences is known as between-subject or inter-individual biological variation (CVG) (4,6).

Medical laboratory results are compared with reference intervals for healthy individuals. However, a result within the reference interval does not guarantee that the result is normal for a specific patient. At this point, RCV, which includes biological variation and analytical variation, is used, which is a significant indicator of the change between the successive test results of the individual (5).

In this study, measurement uncertainty and RCVs of two different autoanalysers (Abbott Architect ci4100 and ci8200) were calculated in Medical Biochemistry Laboratory at University of Health Sciences Turkey, Haydarpaşa Numune Training and Research Hospital. Measurement uncertainty values were compared with the internationally accepted total allowable error limits to evaluate the quality of the results. Measurement uncertainty and the use of RCV together were also evaluated in the assessment of patient results.

Material and Methods

Ethical approval for this retrospective study was obtained from the Ethics Committee of University of Health Sciences, Haydarpaşa Numune Training and Research Hospital (HNEAH-KAEK 2017/KK/94). Informed consent from patients was not required. The Nordtest guide, "top-down" approach, was used to estimate measurement uncertainty. The calculations were performed using internal quality control (IQC) and external quality control (EQC) data.

Statistical Analyses

IQC Data

The original control sera supplied by Architect were studied 2 times a day (08 am and 06 pm) for all parameters to obtain IQC data (uncertainty-analytical process from reproducibility). Each lot has been evaluated and mean values, standard deviation (SD) and coefficient of variation (CV) values for each level were obtained.

EQC Data

EQC data of KBUDEK program were used for the bias calculation.

Calculation of Measurement Uncertainty

Measurement uncertainty calculation was completed in six stages as follows:

1. Definition of measurement

Measured parameter are; albumin, amylase, alanine transaminase (ALT), total bilirubin, direct bilirubin, blood urea nitrogen (BUN), Ca, Cl, creatinine, glucose, K, LDH, CK, lipase, Mg, Na, total protein, P, C-reactive protein (CRP), aspartate transaminase (AST), β-human chorionic gonadotropin (HCG), creatine kinase (CK) -MB (mass) and troponin-I. Serum sample was used for all tests.

2. Calculation of within-laboratory reproducibility (Rw) component of uncertainty

Using the daily IQC data of each test, the mean, SD and CV values, respectively, were calculated as follows:

$$SD = \sqrt{\frac{\sum(xi-x)^2}{n-1}}$$

xi: measurement result, x: mean value, n: number of measurements

CV% = 100*SD/ x

Rw= $\sqrt{[(CV_1)^2+(CV_2)^2+ ....)_{L1}+(CV_1)^2+(CV_2)^2+ ....)_{L2}+(CV_2)^2+ ...)_{L3}]/n}$

CV1: CV% during the period (n times) used for lot 1, CV2: CV% for lot 2

L1: Low, L2: Normal and L3: High level, n: Total number of CV%

3. Calculation of bias component of uncertainty:

The bias can be obtained from certified reference material or EQC data. EQC data were used in this study.

Since it is recommended to use at least 6 attendance data over a given period of time (at least one year) to obtain reliable bias results from EQC data (7), 6 data were used (every 2 months) for CRP and 12 data for all other parameters (once a month).

u(bias) is divided into two as laboratory bias (RMSbias) and uncertainty component for the certified material [u(Cref)].

The calculations were performed accordingly using the following formulas:

$$u(bias) = \sqrt{RMSbias^2 + u(Cref)^2}$$

$$RMS_{bias} = \sqrt{\frac{\sum(bias_i)2}{n}}$$

bias$_i$: bias % value of the test at that period, n: the number of periods of participation in EQC

Calculation of the bias value of the laboratory from the EQC program;

bias$_i$= 100 x |Clab$_i$ - Clab$_{ref}$| / Clab$_{ref}$

Clab$_i$: Laboratory measurement result, Clab$_{ref}$: Average measurement results of laboratories using the same method and device.

u(Cref) = CVR / √nLab

To calculate the CVR, CV% values for all periods given in the EQC reports for the relevant test are summed and divided by the number of periods.

nLab: For each period of participation, the number of laboratories was summed, and the total number was divided by the number of periods.

4. Conversion of components to standard uncertainty u(Rw)

The Rw values obtained in the second step were divided by 2 within the 95% confidence interval (CI) and the standard uncertainty value u(Rw) was obtained (8).

u(Rw) = Rw/2

5. Calculation of combined standard uncertainty, (u$_c$)

$$u_c = \sqrt{u(bias)^2 + u(Rw)^2}$$

6. Calculation of expanded uncertainty (U)

U= k x u$_c$ k=2

The k-value was taken as approximately 2, representing 95% CI (9).

Rilibak limits were taken as the target value, since no limit value was specified for β-hCG in both guidelines. For β-HCG, Rilibak (Germany) has determined acceptable RMSD (% root mean SD) as 14 % (10).

An algorithm including all the above steps in the calculation of measurement uncertainty is presented in Figure 1.

Calculations of Reference Change Value

Tests for which RCV was determined; albumin, amylase, ALT, total bilirubin, direct bilirubin, BUN, Ca, Cl, creatinine, glucose, K, LDH, CK, lipase, Mg, Na total protein, P, CRP, AST, β-HCG, CK -MB (mass) and troponin-I.

In the calculation of RCV, analytical CV (CVA) values obtained using one-year IQC and intra-individual biological variation coefficient (CVI) were used. CVI values of serum samples for all tests were obtained from the Westgard website. RCV for β-HCG was not calculated because the CVI value was not available on the Westgard website. RCV calculation was performed using classical Fraser formula and logarithmic transformation formula.

RCV (Fraser formula) = $Z \times 2^{1/2} \times CVT$

"Z"-value is 1.65 for one-way change in 95% CI while 1.96 for bidirectional change (11). In this study, the Z-value was assumed to be 1.65, since the percentages of change in one direction were calculated.

The steps in the study of Lund et al. (12) were followed in the application of the logarithmic transformation formula to identify significant change in bidirectional.

$$CVT = \sqrt{CVA^2 + CVI^2}$$

Reference change factor up (RCF_up) and reference change factor down (RCF_down) were obtained by using CVT and Z-values.

$RCF_{up} = \exp(Z \times \sqrt{2} \times CVT/100)$

$RCF_{down} = 1/RCF_{up}$

The patient's first test result was multiplied by the RCF_{up} value to determine the significant increase in consecutive test results of the patient. If the second test result was higher than this product result, this increase was considered significant. Likewise, the patient's first test result was multiplied by the RCF_{down} value. If the second test result was lower than the value obtained by this product result, this decrease was considered significant.

The individuality index (II) was used to determine the RCV value to be used to evaluate the significance of the change in successive test results as follows (13). CVI and CVG values were obtained from Westgard's website.

$II = CVI/CVG$

Microsoft office 2010 was used for statistical analysis and graphics design.
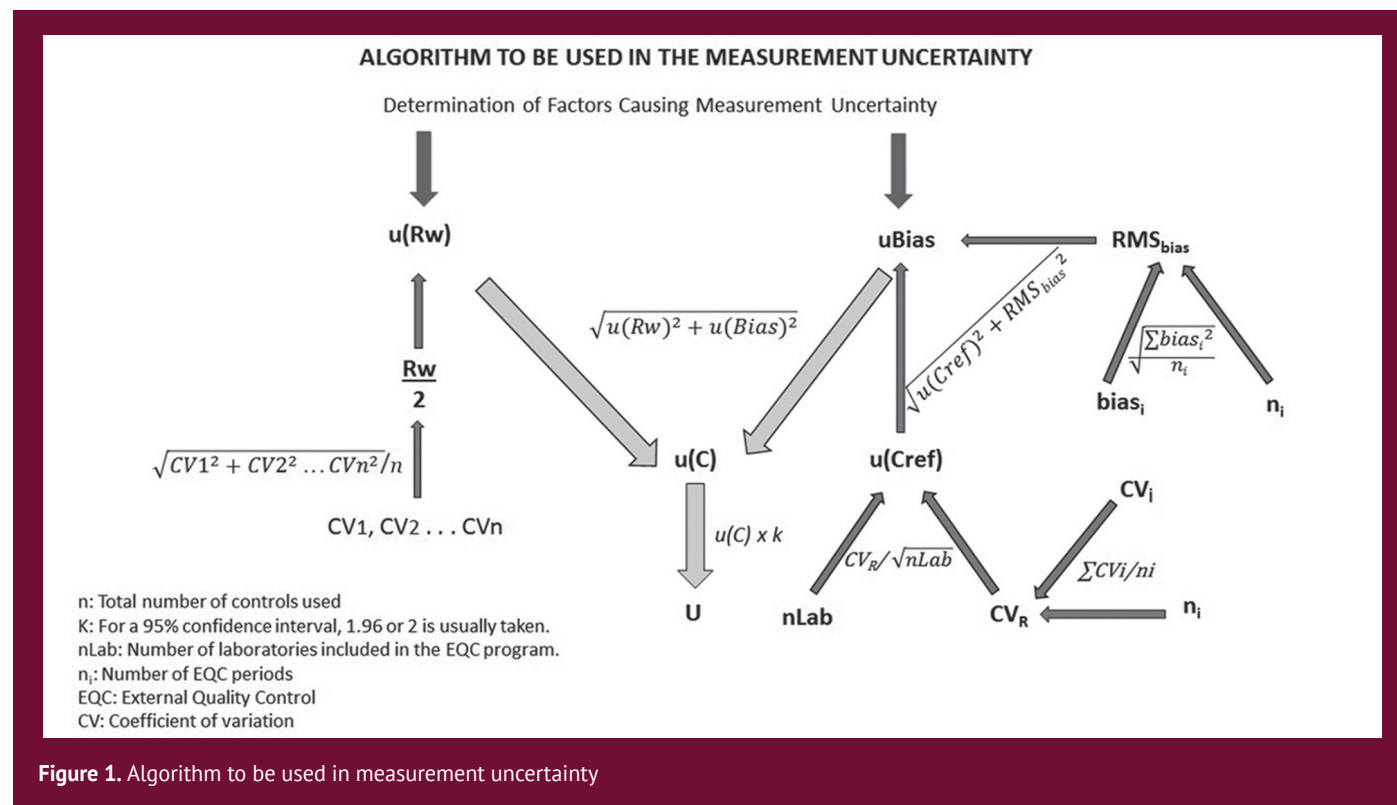
Figure 1. Algorithm to be used in measurement uncertainty

Results

• Results of Measurement Uncertainty

When U-values were examined, the results of the uncertainty obtained from all tests analyzed on our Architect ci4100 and ci8200 devices were within the CLIA'88 total error limits (Table 1, 2). However, it was observed that the uncertainty values determined for albumin, Ca, Cl, Mg, Na and total protein analyzed on both devices did not reach the Westgard total error limits. The uncertainty value for β-HCG analyzed in the Ci8200 autoanalyser was 11.7%. While this value was within the limits, the uncertainty value estimated in the ci4100 instrument was out of the limit (18.78%).

Results of RCV

In the analysis based on the II of amylase, ALT, CK, creatinine, LDH, Mg, total protein, CRP, AST, CK-MB (mass), and troponin-I were <0.6 (Table 3). Accordingly, it was considered that the use of RCV in these tests was more appropriate. On the other hand, it was found that RCV could be used in addition to community-based reference intervals for albumin, total bilirubin, direct bilirubin, urea, calcium, chlorine, glucose, potassium, lipase, sodium and phosphorus with a high II (≥0.6).

The RCV values obtained using both the Fraser method and the logarithmic transformation formula are as shown in Table 4 for all these tests. After applying the logarithmic transformation formula, one-way change values at 95% CI for each test were calculated separately as positive increase and negative decrease.

RCVs of total bilirubin, direct bilirubin, creatinine kinase, lipase and troponin-I tests calculated by logarithmic transformation were higher in increasing direction and lower in decreasing direction than the Fraser method. The RCV calculated according to the Fraser method exceeded 100% in decreasing direction, whereas the RCV calculated using logarithmic transformation was below 100% for CRP parameter (Figure 2).

Discussion

The appropriate application of modern medicine is unlikely without test results which are performed in clinical laboratories. In laboratory, the measurement of these tests is carried out by a series of complex precision instruments and various automated electronic equipment using test procedures. However, no test result is completely certain. These errors and uncertainties in test results may also vary depending on the measurement system, measurement procedure, operator skill, environmental situations and other influencers. Due to this distribution, the concept of uncertainty of measurements was needed to express the uncertainty in numbers (1,14,15). In brief, the measurement uncertainty is the doubt that exists about any measurement results.

The basic assumption in calculating measurement uncertainty is based on providing information for the identification and correction of all systematic errors at early stage of the assessment process. The quality of a measurement is linked to the fact that uncertainty about random and systematic error (bias) is taken into account on the correct basis (16). It therefore includes all factors that affect the interpretation of the value used for diagnosis, treatment, and monitoring of patients. Furthermore, understanding the analytical aspects of the test for each laboratory is possible by defining the measurement uncertainty, which helps in the implementation of good clinical practice and reduces errors (17). Otherwise,
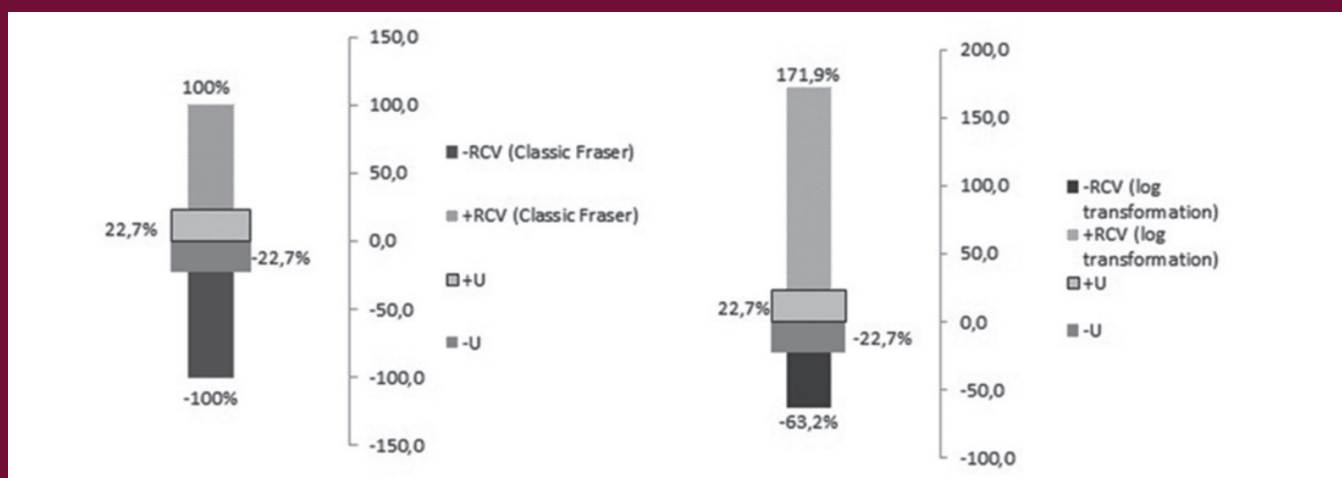
Figure 2. Measurement uncertainty (U) and reference change value (RCV) calculated for CRP. A) RCV by Classical Fraser and U, B) RCV by logarithmic transformation and U

these errors may increase medical costs and cause patients to be misdiagnosed, and even put patients in dangerous situations. Therefore, although measurement uncertainty is relatively new for clinical laboratories, it is an important issue (18). The measurement uncertainty is the datum that ensures the reliability of the result.

Two main approaches are recommended by international guidelines for calculating uncertainty. The bottom-up approach involves a comprehensive examination of measurements. In this approach, each source of uncertainty is determined one by one and the combined uncertainty value is obtained (3). The top-down approach is generally based on the evaluation of EQC data with IQC data. In studies conducted on the calculation of measurement uncertainty of some tests, it has been concluded that the results of measurement uncertainty obtained using both approaches are equivalent and can be used interchangeably (19).

The measurement uncertainty in clinical laboratories according to the down-to-up approach is difficult and time consuming. However, the up to down approach is based on

bias and intra-laboratory reproducibility values. For this reason, clinical laboratories prefer this method, which is more simple in applicability, to calculate measurement uncertainty. Moreover, if the analytical conditions for total imprecision are met, the uncertainty components do not need to be identified and estimated separately unless there is a specific clinical objective. In addition, since the effects of uncertainty sources such as calibrator and reagent changes, technician change, humidity and temperature fluctuations are reflected to the IQC data in the long term, the top-down approach for determining the measurement uncertainty seems more useful for the laboratory (20,21). For these reasons, we used the 6-step Nordtest guide with top-down approach to calculate measurement uncertainty in this study. Adhering to these principles, the U values calculated from all the tests analyzed on our Architect ci4100 and ci8200 devices were within CLIA'88 total error limits, indicating that these values could be used for our laboratory. However, the U values calculated for albumin, Ca, Cl, Mg, Na and total protein were outside the Westgard limits. This finding suggested that improvements

Table 1. Uncertainty components, combined uncertainty and uncertainty of the expanded measurement of tests measured in the ci4100 instrument

Test	RMS Bias	U (Cref)	uBias	U (Rw)	u_c	U (%)	Westgard TEa %	CLIA88 TEa %
ALB	4.34	0.49	4.36	1.83	4.73	9.46	4.07	10
ALT	1.37	0.51	1.46	1.93	2.42	4.84	27.48	20
AMY	5.25	0.42	5.27	1.19	5.40	10.80	14.6	30
AST	1.60	0.37	1.64	1.16	2.01	4.01	16.69	20
D.BIL	4.19	0.64	4.24	3.11	5.25	10.51	44.5	-
T.BIL	4.31	0.74	4.37	2.25	4.92	9.84	26.94	20
BUN	1.27	0.38	1.32	1.87	2.29	4.57	15.55	9
Ca	1.84	0.30	1.86	1.45	2.36	4.73	2.55	10 (±1.0 mg/dL)
CK	3.48	0.51	3.52	1.17	3.71	7.42	30.3	30
Cl	1.03	0.22	1.05	0.75	1.29	2.57	1.5	5
CREA	1.88	0.34	1.92	1.29	2.31	4.62	8.87	15
GLU	2.39	0.33	2.41	1.20	2.70	5.39	6.96	10
K	1.01	0.25	1.04	0.75	1.28	2.56	5.61	12.5 (±0.5 mmol/L)
LDH	2.72	0.46	2.76	1.29	3.05	6.10	11.4	20
Lipase	7.02	1.14	7.11	3.17	7.79	15.57	37.88	-
Mg	2.87	0.73	2.96	1.70	3.41	6.82	4.8	-
Na	0.88	0.19	0.90	0.62	1.09	2.18	0.73	2.85 (±4 mmol/L)
P	1.33	0.37	1.38	1.68	2.17	4.35	10.11	-
TP	2.74	0.35	2.76	1.37	3.08	6.17	3.63	10
CRP	3.72	1.71	4.10	3.51	5.39	10.79	56.6	-
β-HCG	8.73	0.78	8.77	3.35	9.39	18.78	-	-
CK-MB	8.26	1.94	8.49	3.78	9.29	18.58	30.06	-
Tp-I	4.96	1.62	5.21	3.49	6.27	12.55	27.91	-

CLIA'88: The Clinical Laboratory Improvement Amendments of 1988, TEa: Total allowable error, ALT: Alanine transaminase, AST: Aspartate transaminase, BUN: Blood urea nitrogen, CI: Confidence interval, LDH: Lactate dehydrogenase, CRP: C-reactive protein, HCG: Human chorionic gonadotropin, CK: Creatine kinase

should be made to reduce the sources of error for these tests. For albumin test, U values in Architect ci8200 instruments were found as 5.98%. This value was consistent with the study performed by Bal et al. (18) on three different devices for albumin (U= 7.35%, 6.49% and 6.47%, respectively). However, this value was higher than the U value (3.4%) determined by Iqbal et al. (22) for albumin. The U values (9.46%) determined for the albumin test in Architect ci4100 device were well above these data of two researches.

When some of the results obtained in our study were compared with previous studies, it was observed that U values (4.73% and 5.25%) found in Architect ci4100 and ci8200 devices for Ca test were much higher than those found for plasma samples (0.12%) by Padoan et al. (23) However, it was found that U values (2.57% and 2.86%) found in Architect ci4100 and ci8200 devices for Cl test were lower than those determined by Padoan et al. (23) (3.76%). For the Mg test, U values (6.82% and 11.36%) found in Architect ci4100 and ci8200 devices were higher than those determined by Iqbal et al. (22) (4%) for their devices. The U values for the Na test in Architect ci4100 and ci8200 devices were 2.18% and 2.66% respectively, while

Bal et al. (18) found that U values for the same analyte were close to our values as 2.32%, 2.21% and 2.07%, respectively. Padoan et al. (23) found that U value for Na was 1.81%. For the total protein test, the U values of Architect ci4100 and ci8200 devices were 6.17% and 5.39%, respectively, whereas Bal et al. (18) obtained U values for their 3 different devices as 8.40, 8.51% and 8.39% respectively. Iqbal et al. (22) found the uncertainty value of 4.7% for total protein. According to all the above data, we had better performance compared to Bal et al. (18), worse than Iqbal et al. (22) and different analyte-based performances with Padoan et al. (23).

When we examine our uncertainty results in detail, a bias error usually has a greater share. As a laboratory, we make our accuracy according to our results in the EQC program. However, the concentration of the analyte in the EQC sample is not given. Instead, the average of the measurement results of all laboratories participating in the EQC program is considered the real value. Therefore, this value also includes the errors of each laboratory from the analytical process during the measurement. As the number of laboratories participating in the EQC program increase,

Table 2. Uncertainty components, combined uncertainty and uncertainty of the expanded measurement of tests measured in the ci8200 instrument

Test	RMS Bias	U (Cref)	U bias	U (Rw)	u_c	U (%)	Westgard TEa%	CLIA88 TEa%
ALB	2.29	0.49	2.34	1.86	2.99	5.98	4.07	10
ALT	2.46	0.51	2.51	1.73	3.05	6.10	27.48	20
AMY	4.34	0.42	4.36	1.41	4.58	9.16	14.6	30
AST	2.21	0.37	2.24	1.42	2.65	5.30	16.69	20
D.BIL	2.90	0.64	2.97	3.16	4.34	8.68	44.5	-
T.BIL	4.65	0.72	4.71	2.96	5.56	11.12	26.94	20
BUN	2.05	0.37	2.09	2.75	3.45	6.90	15.55	9
Ca	1.97	0.29	1.99	1.71	2.62	5.25	2.55	10 (±1.0 mg/dL)
CK	2.96	0.51	3.00	1.52	3.37	6.74	30.3	30
Cl	1.19	0.22	1.21	0.76	1.43	2.86	1.5	5
CREA	2.08	0.34	2.10	1.63	2.66	5.32	8.87	15
GLU	1.71	0.33	1.74	1.26	2.15	4.30	6.96	10
K	1.03	0.25	1.06	0.78	1.32	2.64	5.61	12.5 (±0.5 mmol/L)
LDH	4.34	0.45	4.36	1.90	4.76	9.51	11.4	20
Lipase	6.30	1.14	6.41	2.96	7.05	14.11	37.88	-
Mg	5.21	0.73	5.26	2.16	5.68	11.36	4.8	-
Na	1.11	0.19	1.12	0.71	1.33	2.66	0.73	2.85 (±4 mmol/L)
P	2.03	0.37	2.06	2.12	2.95	5.91	10.11	-
TP	2.24	0.35	2.26	1.46	2.69	5.39	3.63	10
CRP	10.43	1.71	10.57	4.10	11.34	22.68	56.6	-
β-HCG	4.63	0.78	4.69	3.51	5.86	11.72	-	-
CK-MB	4.88	1.94	5.25	3.34	6.23	12.45	30.06	-
Tp-I	7.79	1.62	7.96	3.66	8.76	17.52	27.91	-

CLIA'88: The Clinical Laboratory Improvement Amendments of 1988, TEa: total allowable error, ALT: Alanine transaminase, AST: Aspartate transaminase, BUN: Blood urea nitrogen, CI: Confidence interval, LDH: Lactate dehydrogenase, CRP: C-reactive protein, HCG: Human chorionic gonadotropin, CK: Creatine kinase

the errors of the laboratories from this analytical process decrease to insignificant levels. Thus, it is accepted that the measurement results of the laboratories participating in the EQC program are remarkably close to the actual value. It is not recommended to use imprecision to estimate the measurement uncertainty resulting from the performance of laboratories in the EQC program when participation in the EQC program is low because there is generally less data for estimating uncertainty (20).

Another important factor affecting the EQC results and therefore the size of the bias result is that the samples are in lyophilized state (24). Therefore, the bias values of all tests belonging to the period in which the pipetting error during the reconstitution phase has been made will tend to come up high. The suggestion of glass pipettes in the reconstitution of such samples reduces the pipetting error but does not completely eliminate it. The best way to minimize bias resulting from the reconstitution of lyophilized samples is to use liquid samples instead of lyophilized samples for EQC, or

to use standard dilution samples with lyophilized samples. In the light of this information, we think that our U values may be higher depending on the lyophilized EQC samples.

In the evaluation of the test results of individuals, reference intervals which are determined by age, gender and other variables that are not very compatible for themselves are used. This allows data to be used only in a superficial manner. However, it does not consider the basic information about reference intervals and the individual and inter-individual factors of the analytes. Therefore, it is controversial to evaluate the individual successive test results according to population-based reference intervals (25,26). Because, although there is no change (improvement or deterioration) in patients' current health status, one of the patients' consecutive results may be within the reference interval and the other may be out of the reference interval.

The reason for the changes observed in a person's successive test results may be due to the improvement of his clinical condition or vice versa, and was mostly attributed to CVI and CVA variables

Table 3. CVI, CVG, II, CVA and CVT values for all tests					
Test	CVI	CVG	II	CVA	CVT
ALB	3.20	4.75	0.67	3.72	4.906
AMY	8.7	28.3	0.31	2.80	9.140
ALT	19.4	41.6	0.47	3.47	19.707
T.BIL	21.8	28.4	0.77	5.72	22.538
D.BIL	36.8	43.2	0.85	6.33	37.341
UREA	12.1	18.7	0.65	4.92	13.062
Ca	2.1	2.5	0.84	3.42	4.017
CK	22.8	40	0.57	3.05	23.003
Cl	1.2	1.5	0.80	1.52	1.940
CREA	5.95	14.7	0.40	3.26	6.782
GLU	5.6	7.5	0.75	2.52	6.142
K	4.6	5.6	0.82	1.56	4.858
LDH	8.6	14.7	0.59	3.80	9.401
Lipase	32.2	31.8	1.01	5.92	32.740
Mg	3.6	6.4	0.56	4.32	5.623
Na	0.6	0.7	0.86	1.41	1.534
TP	2.75	4.7	0.59	2.92	4.010
P	8.15	10.8	0.75	4.23	9.185
CRP	42.2	76.3	0.55	8.19	42.988
AST	12.3	23.1	0.53	2.85	12.626
CK-MB (mass)	18.4	61.2	0.30	7.13	19.732
Tp -I	14.05	63.75	0.22	7.31	15.840

II: Individuality index. If II <0.6, RCV is used. If II >1.4, the reference interval is used. If 0.6< II <1.4, both are recommended to be used together. CVI: Intra-individual biologic variation, CVG: Inter-individual biologic variation, CVT: Total CV value, ALT: Alanine transaminase, AST: Aspartate transaminase, CI: Confidence interval, LDH: Lactate dehydrogenase, CRP: C-reactive protein, CK: Creatine kinase

Table 4. RCV values calculated by applying classical fraser and logarithmic transformation			
Test	Class fraser RCV (%)	Log. trans (%) (+)	Log. trans (%) (-)
ALB	11.45	12.09	-10.79
AMY	21.33	23.69	-19.16
ALT	45.99	58.17	-36.78
T.BIL	52.59	68.94	-40.81
D.BIL	87.13	138.39	-58.05
UREA	30.48	35.51	-26.21
Ca	9.37	9.80	-8.92
CK	53.68	70.77	-41.44
Cl	4.53	4.62	-4.41
CREA	15.83	17.09	-14.60
GLU	14.33	15.36	-13.31
K	11.34	11.97	-10.69
LDH	21.94	24.45	-19.65
Lipase	76.40	114.19	-53.31
Mg	13.12	13.97	-12.26
Na	3.58	3.63	-3.51
TP	9.36	9.78	-8.91
P	21.43	23.82	-19.24
CRP	100.31	171.86	-63.22
AST	29.46	34.14	-25.45
CK-MB (mass)	46.04	58.26	-36.81
TROP -I	36.96	44.56	-30.82

The RCV was calculated as a one-way change in the 95% confidence interval. ALT: Alanine transaminase, AST: Aspartate transaminase, CI: Confidence interval, LDH: Lactate dehydrogenase, CRP: C-reactive protein, CK: Creatine kinase

(27). RCV can be detected using these variables. Although two-way change is generally used in RCV calculations, according to Cooper et al. (28), one-way change is more appropriate. Fraser also made similar recommendations when evaluating sequential troponin measurements in the assessment of acute cardiac cases (28). Therefore, we made our calculation based on one-way change. Since the analytical CV values of almost all tests on the Architect ci8200 device are higher than on the Architect ci4100, the CVA values of our Architect ci8200 device, which represent a wider range in RCV calculations, were based on.

The II determines whether the reference interval or RCV is preferred for the evaluation of individual test results. The use of population-based reference interval is not considered appropriate when the II is less than 0.6 because this reference interval will cover a very few individuals and will provide very limited benefit in assessing whether there is a significant change in results (29). Therefore, it would be more appropriate to compare the results with the previous basal results. For tests with an II greater than 1.4, reference interval should be preferred. It is reported that RCVs as well as reference intervals are more suitable for the evaluation of tests with an II less than 1.4 (4).

In a study conducted by Ko et al. (30), they calculated the RCV values as bidirectional at 95% CI according to the classical Fraser method. RCV values were found to be 9.47% for albumin, 5% for ALT, 24.2% for amylase, 34.4% for AST, 33.7% for BUN, 6.7% for calcium, 4.3% for Cl, 18% for creatinine, 102.1% for direct bilirubin, 15.8% for glucose, 12.9% for K, 24% for LDH, 3.2% for Na, 7.9% for total protein, 22.7% for P, and 60.5% for total bilirubin; respectively (30). These values were close to almost all our values except direct bilirubin. The decreasing RCV value determined by Ko et al. (30) for bilirubin was unusable because it exceeded 100%. However, in our study, we found that the RCV value for the direct bilirubin in the decreasing direction with the classical Fraser approach was less than 100% and the RCV value calculated with the logarithmic transformation approach was -58.05%. As can be seen from this, it is not possible to use the Fraser approach in the decreasing direction, while the clinical use of the value determined by the logarithmic transformation approach is possible.

The RCV values obtained by Walz and Fierz by applying logarithmic transformation were close to the RCV values calculated in our study. There is a significant difference between only the RCV results of troponin-I test (140% and - 58%) (31). In our study, a narrower range was found for troponin-I test. This difference was probably attributable to the difference in analytical performance between laboratories and the type of sample used for troponin-I measurement. These researchers used plasma CVI values for troponin-I. Since the sample matrix, which is most suitable for analyzing, can be evaluated by the smallness of the CVI value, these researchers have obtained

a larger RCV value, probably due to plasma use. However, we used serum samples in our study. In both studies, the reason for reaching similar results for many tests was attributed to the similar biological variation values as well as the close analytical performance of the instruments and kits.

Normally, the intra-individual biological variation shows a random fluctuation due to the homeostatic fluctuations of the individual (homeostatic set points). In addition, wider biological fluctuations will be observed in abnormal metabolic conditions such as disease, drug use, pregnancy and menopause. Therefore, in determining RCV values, it is considered that it is more appropriate to use the classical Fraser method for normal distribution tests and logarithmic transformation method for non-normal distribution tests. Furthermore, it is not possible to use RCV values calculated by the Fraser method above 100% in the direction of decrease, because such a reduction is not possible for repeated tests. In particular, it is considered that the logarithmic conversion formula should be preferred for tests that do not show normal distribution and have a wide biological variation (CRP, direct bilirubin, total bilirubin, troponin, creatinine kinase) (4,6). In summary, since a significant proportion of individuals applying to hospitals consist of individuals with disease, drug use or abnormal metabolic conditions, laboratories should be able to calculate RCV values using both classical Fraser and logarithmic transformation and keep this information available.

Conclusion

Each laboratory should calculate MU values to bring the reliability of test results close to international limits. Logarithmic transformation formulas should be used in the RCVs calculation of tests with high biological variation, such as CRP. In addition, MU and RCV should be given with the test results to improve diagnostic accuracy.

Ethics

Ethics Committee Approval: Ethical approval for this retrospective study was obtained from the ethics committee of University of Health Sciences, Haydarpaşa Numune Training and Research Hospital (HNEAH-KAEK 2017/KK/94).

Informed Consent: Informed consent from patients was not required.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Project Development: F.Ö., A.K., M.M.Y., Data Collection or Processing: H.H.P., E.S., Ş.K., M.Z.Ç., A.K., M.M.Y., Analysis or Interpretation: F.Ö., Manuscript Editing: A.K., M.M.Y., H.H.P., E.S., Ş.K., M.Z.Ç., Writing: F.Ö.

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Oncogenic Mutation Frequencies in Lung Cancer Patients

Akciğer Kanseri Hastalarında Onkojenik Mutasyon Sıklıkları

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ABSTRACT

Background: The data on mutation frequencies in patients with lung cancer are limited in Turkey. We aimed to determine the frequencies of *EGFR*, *ALK*, *ROS1*, and *BRAF* gene mutations in patients with lung cancer in this study.

Materials and Methods: Data of 329 patients with lung cancer, who underwent molecular examination at the İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Medical Pathology Laboratory, were analyzed retrospectively.

Results: A total of 329 patients with lung cancer, of whom 257 were male and 71 were female, were included in the study. The average age of women was 65.7±11.2 years and the average age of men was 64.6±10.3 years. Thirty nine patients (11.8%) were non-smokers. The prevalence of smoking was 97.2% (n=251) in male patients and 54.9% (n=39) in female patients. Two hundred sixty one (79.3%) of the patients had adenocarcinoma, 50 (15.2%) squamous cell lung cancer, 13 (3.9%) non-small cell lung cancer, 4 (1.3%) small cell lung cancer, 1 patient (0.3%) was diagnosed with large cell lung cancer. Gene mutation was detected in 52 patients (15.8%). EGFR mutation was detected in 32 patients (9.7%), ALK in 17 patients (5.5%), ROS1 in 2 patients (0.6%), and BRAF mutation in 1 patient (0.3%). The frequency of mutation was 30.7% (n=12) in non-smoking patients, and 18.2% (n=53) in smokers. The most common genetic alteration was deletions in the *EGFR* gene in exon 19.

Conclusion: In our study, we found the frequencies of EGFR and ALK mutations similar to the studies conducted around the world but BRAF and ROS1 mutations frequencies were lower compared to studies conducted around the world. In addition, we found that all mutation frequencies were lower than in studies conducted in our country. We thought this was related to the low number of cases in the studies and more selective patient selection.

Keywords: Lung cancer, oncogenic mutations, EGFR, ALK, ROS1, BRAF

ÖZ

Amaç: Ülkemizde akciğer kanseri hastalarında mutasyon sıklıkları ile ilgili veriler sınırlıdır. Çalışmamızda akciğer kanserli hastalarda *EGFR, ALK, ROS1, BRAF,* gen mutasyonlarının sıklıklarının belirlenmesi amaçlanmıştır

Gereç ve Yöntemler: İstanbul Sultan 2. Abdülhamid Han Eğitim Araştırma Hastanesi, Tıbbi Patoloji Kliniği Laboratuvarı'nda moleküler incelemeye alınan, akciğer kanseri tanılı toplam 329 hastanın verileri retrospektif olarak incelendi.

Bulgular: Akciğer kanseri tanılı 258'i erkek ve 71'i kadın toplam 329 hasta çalışmaya dahil edildi. Kadın hastaların ortalama yaşı 65,7±11,2, erkek hastaların ortalama yaşı 64,6±10,3 idi. 39 hasta (%11,8) hiç sigara kullanmamıştı. Erkek hastalarda sigara kullanma sıklığı %97,2 (n=251), kadın hastalarda sigara kullanma sıklığı %54,9 (n=39) idi. Hastaların 261'i (%79,3) adenokanser, 50'si (%15,2) squamoz hücreli akciğer kanseri, 13'ü (%3,9) küçük hücreli dışı akciğer kanseri, 4'ü (%1,3) küçük hücreli akciğer kanseri, 1 hasta (%0,3) da büyük hücreli akciğer kanseri tanılı idi. Elli iki hastada (%15,8) gen mutasyonu saptandı. Otuz iki hastada (%9,7) EGFR, 17 hastada (%5,5) ALK, 2 hastada (%0,6) ROS1, 1 hastada (%0,3) BRAF mutasyonu saptandı. Sigara içmeyen hastalarda mutasyon sıklığı %30,7 (n=12), sigara içenlerde mutasyon sıklığı %18,2 (n=53) bulundu. Sigara içmeyen hastalarda 10 hastada EGFR (%25,6), 2 hastada (%5,1) ise ALK mutasyonu pozitif saptandı. En sık saptanan genetik değişiklik *EGFR* geninde ekzon 19'da saptanan delesyonlardı.

Sonuç: Çalışmamızda EGFR ve ALK mutasyon sıklıklarını dünyada yapılan çalışmalarla benzer bulduk. Ancak BRAF ve ROS1 mutasyon sıklıkları dünyada yapılan çalışmalara göre daha düşüktü. Ayrıca tüm mutasyon sıklıklarının ülkemizde yapılan çalışmalara göre daha düşük olduğunu saptadık. Bu durumun yapılan çalışmalardaki düşük olgu sayısı ve daha selektif hasta seçimi ile ilgili olabileceğini düşündük.

Anahtar Kelimeler: Akciğer kanseri, onkojenik mutasyonlar, EGFR, ALK, ROS1, BRAF

Introduction

Lung cancer is still the leading cause of cancer deaths worldwide (1). There are 1.8 million new cases with lung cancer and 1.6 million people die due to lung cancer per year (2). Non-small cell lung cancers (NSCLC) are 80% of lung cancers (3). Since the majority of cases are diagnosed at an advanced stage, the mortality is high. The standard chemotherapy regimens applied so far have not had a dramatic effect on the prognosis (5-year survival 15%) (4). In recent years, a better understanding of the pathogenic genomic changes of NSCLC and determination of molecular tests and biomarkers used to identify patients with these genomic changes have enabled the use of molecular target therapies and immunotherapy in advanced stage patients with NSCLC, especially for adenocarcinoma. Pharmacological treatments guided by epidermal growth factor receptor (EGFR), anaplastic lymphoma kinase (ALK), proto-oncogene receptor tyrosine kinase 1 (ROS1) and V-Raf murine sarcoma viral oncogene homolog B (BRAF) driver mutations have opened a new era in the treatment of advanced lung cancer. Although many mutations have been identified in lung cancer, the mutation status is still unknown in more than 50% of patients with lung cancer and therapeutic targets can be determined only in 20% of the patients (5).

The detection of EGFR, ALK, ROS1 and BRAF in patients with advanced stage or metastatic adenocarcinoma and NSCLC, whose histologically clear distinction cannot be made, and in patients with squamous cell carcinoma and no history of smoking is currently recommended (6). Therefore, adequate biopsies are needed to perform molecular examination along with histopathological diagnosis.

The data on mutation frequencies in patients with lung cancer are limited in Turkey. We aimed to determine the frequencies of *EGFR*, *ALK*, *ROS1*, *BRAF* gene mutations in patients with lung cancer in this study.

Material and Methods

A total of 329 patients with lung cancer were included in the study. The samples were performed molecular examination at the İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital, Clinic of Medical Pathology Laboratory between 2017 and 2019. Ethics committee approval of the study was obtained (number: 57 and date: 18/03/2020). Preoperative approvals were obtained from all patients. Age, gender, smoking history and histopathological diagnoses of the patients were recorded. For *EGFR* gene exon 18, 19, 20, 21 and *BRAF* gene exon 15 mutation analyses, the area containing the most tumor cells in the Hematoxylin & Eosin (H&E) stained section was determined. Tumor areas determined from 10 unstained histological tissue sections of 10 μm thicknesses were manually macro dissected with a scalpel. After standard deparaffinization and hydration, DNA was isolated with the QIAamp FFPE Tissue kit. DNA concentration of the samples was measured spectrophotometrically (nanodrop). The 18th, 19th, 20th, 21st exons of the *EGFR* gene and the 15th exons of the BRAF gene were amplified by polymerase chain reaction (PCR) in the thermal cycler using the HotStar Taq DNA Polymerase kit and appropriate primers. The sample was run in pairs with positive, negative and noDNA template controls. Density and contamination control of PCR products was done by gel electrophoresis process. PCR products were purified with the QIAquick PCR Purification kit. Two-way (forward and reverse) DNA sequence analysis was performed on the purified amplicons using the Big Dye Terminator v3.1 Cycle Sequencing kit with the Sanger sequence technique. After ethanol precipitation, PCR products were run on the ABI-3730 (48 capillary) automatic sequencing device. The resulting electropherograms were visually evaluated using SeqScape Software 3.0 by comparing them with the reference sequences NM_005228.3 (*EGFR* gene) and NM_004333.4 (*BRAF* gene), together with their positive and negative controls.

In order to investigate the *ALK* and *ROS1* genes by FISH technique, the area containing the most tumor cells in the H&E stained section was determined. After the macrodissection of the 2 μm thick section taken from the formal-fixed paraffin embedded block, it was prepared by standard prehybridization steps. "ZytoLight SPEC ROS1 Dual Color Break Apart Probe, Z-2144-200, Zytovision" (proximal part of the breakpoint region of the *ROS1* gene (6q22.1): green spectrum, distal part: orange spectrum) and reconstruction in *ALK* gene for evaluation of rearrangement in the *ROS1* gene; using "ZytoLight SPEC ALK Dual Color Break Apart Probe, Z-2124-200, Zytovision" (proximal part of the breakpoint region of the *ALK* gene (2p23.1-p23.2):

green spectrum, distal part: orange spectrum) for the evaluation of regulation, standard FISH process was applied. One hundred tumor cells were evaluated using Leica DM 2.500 fluorescence microscope and Argenit Akas imaging system.

Statistical Analysis

No statistical analysis was needed.

Results

A total of 329 patients with lung cancer, of whom 257 were male and 71 were female, were included in the study. The average age of the patients was 64.9±10 years. The mean age of women was 65.7±11.2 years and the mean age of men was 64.6±10.3 years. 39 patients (11.8%) were non-smokers. The prevalences of smoking of male and female patients were 97.2% (n=251) and 54.9% (n=39), respectively. Two hundred sixty one (79.3%) of the patients were diagnosed as adenocarcinoma, 50 (15.2%) of the patients were diagnosed as squamous cell lung cancer (SCC), 13 (3.9%) of the patients were diagnosed as NSCLC, 4 (1.3%) of the patients were diagnosed as small cell lung cancer (SCLC) and 1 patient (0.3%) was diagnosed as large cell lung cancer (Table 1). Gene mutation was detected in 52 patients (15.8%). EGFR mutation was detected in 32 patients (9.7%), ALK was positive in 17 patients (5.5%), ROS1 was positive in 2 patients (0.6%), and BRAF mutation was positive in 1 patient (0.3%). Thirty of the patients, whose biopsies were positive for EGFR, were diagnosed as adenocarcinoma, 1 patient was diagnosed as SCLC (transformed from adenocarcinoma) and 1 patient was diagnosed as SCC. One of the patients with positive ALK mutation was diagnosed as SCC and 16 were diagnosed as adenocarcinoma. All of the patients with positive ROS1 and BRAF mutations were diagnosed as adenocarcinoma (Table 2). The frequency of mutation was 30.7% (n=12) in non-smoking patients, and 18.2% (n=53) in smokers. EGFR (25.6%) was positive in 10 and ALK mutation was positive in 2 of the non-smoking patients (5.1%). The most common genetic alteration was deletions in the *EGFR* gene in exon 19 (Table 3).

Discussion

Genomic changes and mutations in lung cancer vary in different populations. While the incidence of EGFR, ALK, ROS1 and BRAF mutations in patients with adenocarcinoma is approximately 30% in the Uninted States of America, this rate is 60% in Japan (7,8). Çalıskan et al. (9) detected at least one gene mutation in 37 (46.2%) of 80 patients with lung cancer in a Turkish population. Bilgin et al. (10) detected at least one gene mutation in 60 (22.9%) of 260 NSCLC patients and EGFR was positive in 38 (14.6%), ALK was positive in 20 (7.69%), and ROS1 was positive in 2 (0.76%) of them. In our study, we found at least one gene

mutation in 15.8% (n=52) of the patients. High mutation detection rate in the studies of Çalıskan et al. (9) and Bilgin et al. (10) may be related to the low number of patients and the more specific patient selection.

Although the true incidence of EGFR mutation in lung cancers is unknown, the incidence varies between 0 and 13% (11,12). EGFR mutation is more common in female non-smoking patients with adenocarcinoma. In our study, the frequency of EGFR mutation was found to be 9.7%, and 93.7% (n=30) of the patients with positive EGFR mutation had adenocarcinoma. One patient was diagnosed as SCC, and the diagnosis of one patient was transformed from adenocarcinoma to small cell lung cancer. Similar to the literature, the frequency of EGFR mutations was higher in patients who never smoked (n=10, 25.6%).

The frequency of ALK rearrangement in NSCLC is between 4% and 5% (13). In our country, Bilgin et al. (10) reported that 20 (7.69%) of 260 NSCLC patients were positive for ALK mutation, Seymen and Gümüşlü (14) detected ALK rearrangement in 8 (16%) of 14 50 NSCLC patients. Aytekin (15) detected ALK rearrangement in 2 (5.1%) of 130 lung cancer patients, 36 of whom had adenocarcinoma. In our study, the frequency of ALK rearrangement was 5.5%, similar to the literature. The frequency of ALK rearrangement was higher in female patients (n=6, 8.4%).

The frequency of ROS1 rearrangement in NSCLCs varies between 1% and 2% (16). ROS1 rearrangement is observed more frequently in young and non-smoking patients. In a limited number of studies on the frequency of ROS1 rearrangement in Turkey, the frequency varies between 0% and 0.76% (10,15). In our study, in contrast to the literature, ROS1 rearrangement was detected in 2 male patients (0.6%) over 65 years of age with a history of smoking and they were diagnosed as adenocarcinoma.

BRAF mutations are observed in 1-3% of patients with NSCLC. It is observed more frequently in patients with a smoking history. Dogan et al. (17) detected BRAF mutation in 1 (2.38%) of 42 patients with advanced NSCLC in their study. Çalıskan et al. (9) detected BRAF mutation in 1 patient (1.25%) of 80 NSCLC patients. In our study, BRAF mutation was found in 1 male patient (0.3%) with an active smoking and diagnosis of adenocarcinoma.

Conclusion

In our study, we found the frequencies of EGFR and ALK mutations similar to the studies conducted around the world but the frequencies of BRAF and ROS1 mutations were lower compared to studies conducted around the world. In addition, we found that all mutation frequencies were lower than in studies conducted in our country. We thought this was related to the low number of cases in the studies and more selective patient selection. Our study is one of the largest series of studies on

Table 1. Clinical characteristics of the lung cancer patients				
Variables		Female (n=71) (21.6%)	Male (n=258) (78.4%)	Total (n=329) (100%)
Age	Mean (SD) (years)	65.7±11.2	64.6±10.3	64.9±10
	(Min-max)	34-89	32-89	32-89
Smoking	Yes	39 (54.9)	251 (97.2)	290 (88.2)
	No	32 (45.1)	7 (2.8)	39 (11.8)
Histology				
Adenocarcinoma		64 (90.2%)	197 (76.3%)	261 (79.3%)
Squamous cell carcinoma		2 (2.8%)	48 (18.6%)	50 (15.2%)
Non-small cell carcinoma		4 (5.6%)	9 (3.49%)	13 (3.9%)
Small cell carcinoma		1 (1.4%)	3 (1.2%)	4 (1.3%)
Large cell carcinoma		-	1 (0.41%)	1 (0.3%)
SD: Standard deviation, min: Minimum, max: Maximum				

Table 2. Mutation frequencies according to lung cancer histologic types						
Gene	Histology of lung cancer					
	Adenocarcinoma (n=261)	Squamous cell carcinoma (n=50)	Non-small cell carcinoma (n=13)	Small cell carcinoma (n=4)	Large cell carcinoma (n=1)	Total (n=329)
EGFR	30 (11.5%)	1 (2%)	-	1* (25%)	-	32 (9.7%)
ALK	16 (6.1%)	1 (2%)	-	-	-	17 (5.5%)
ROS1	2 (0.76%)	-	-	-	-	2 (0.6%)
BRAF	1 (0.38%)	-	-	-	-	1 (0.3%)
*: Transformation from adenocarcinoma, EGFR: Epidermal growth factor receptor, ALK: Anaplastic lymphoma kinase, ROS1: Poto-oncogene receptor tyrosine kinase 1, BRAF: V-Raf murine sarcoma viral oncogene homolog B						

Table 3. Molecular alterations determined in the study		
Gene	Molecular subtypes	Molecular alterations
EGFR	Exon 18	c.2155G>T
	Exon 19	c.2237_2255>T, c.2239_2248TTAAGAGAAG>C, c.2235_2249del15, c.2239_2256del18, c.2240_2257del18, c.2239_2253del15, c.2240_2254del15, c.2237_2251del15, c.2237_2252delinsT, c.2239_2248delinsC, p.L747_A750de
	Exon 20	c.2311_2312insGCGTGGACA, c.2313_2314insACG, c.2369C>T, c.2296_2297insTGGCCAGCG, c.2464G>A
	Exon 21	c.2573T>G, c.2582 T>A
ALK	EML4-ALK	rearrangement in 2q23 region
ROS1	ROS1	rearrangement in 6q22 region
BRAF	Exon 15	c.1799T>A
EGFR: Epidermal growth factor receptor, ALK: Anaplastic lymphoma kinase, ROS1: Poto-oncogene receptor tyrosine kinase 1, BRAF: V-Raf murine sarcoma viral oncogene homolog B		

mutation frequencies in lung cancers in Turkey. For this reason, it is of great importance to reveal mutation frequencies with more molecular studies in our country and to popularize the treatments for this.

Ethics

Ethics Committee Approval: Ethics committee approval of the study was obtained (number: 57 and date: 18/03/2020).

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Ayten et al. Oncogenic Mutations Frequencies in Lung Cancer

Informed Consent: Preoperative approvals were obtained from all patients.

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Authorship Contributions

Concept: Ö.A., T.Ç., K.C., O.O., Design: Ö.A., T.Ç., L.E., O.O., Data Collection or Processing: Ö.A., T.Ç., K.C., N.K.T., L.E., Analysis or Interpretation: Ö.A., T.Ç., K.C., N.K.T., L.E., Literature Search: Ö.A., T.Ç., K.C., O.O., Writing: Ö.A., T.Ç.

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A Simple Independent Predictor of in-Hospital and Long-Term Outcomes in Patients with STEMI: Plateletcrit

STEMI Hastalarında Hastane İçi ve Uzun Dönem Olaylar için Basit ve Bağımsız Bir Prediktör: Plateletkrit

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ABSTRACT

Background: Platelet mass, namely plateletcrit (PCT), is the total platelet mass which is calculated by multiplying platelet count with mean platelet volume. There are several studies revealing that higher PCT is associated with higher mortality in patients with myocardial infarction. We aimed to investigate the association of high PCT levels and in -hospital and long-term outcomes of ST-segment elevation myocardial infarction (STEMI) in a high-volume center.

Materials and Methods: A total of 2.415 patients were eligible to the study. The study population was divided into three tertiles according to the PCT values starting from the lowest to highest level (T1,T2,T3). In-hospital and long-term outcomes were compared between these tertiles.

Results: In-hospital acute kidney injury, recurrent myocardial infarction, target lesion revascularization, stent thrombosis, cardiogenic shock and all-cause mortality rates were significantly higher in T3 compared to other tertiles. Besides, the rate of long-term all-cause mortality was higher in T3 than in the other tertiles. In the multivariable regression analysis, high PCT was found as an independent predictor for mortality.

Conclusion: The current study demonstrated the higher in-hospital and long-term unfavorable outcomes in patients with STEMI, who had a high PCT.

Keywords: Myocardial infarction, plateletcrit, mortality

ÖZ

Amaç: Plateletkrit (PCT) olarak bilinen total platelet kütlesi platelet sayısı ile ortalama platelet hacminin çarpılması ile hesaplanır ve bazı akut miyokart enfarktüsü çalışmalarında yüksek PCT değerleri artmış mortalite ile ilişkilendirilmiştir. Biz bu çalışmamızda yüksek hasta hacimli bir merkezdeki ST-segment yükselmeli miyokart enfarktüsü (STYME) hastalarındaki PCT'nin hastane içi ve uzun dönem mortalite ile ilişkisini araştırdık.

Gereç ve Yöntemler: Toplam 2,415 hasta çalışma için uygun bulundu. PCT değerlerine göre hastalar en düşükten en yükseğe doğru sıralanarak 3 tertile ayrıldı (T1,T2,T3). Hastane içi ve uzun dönem sağkalımlar bu gruplar arasında karşılaştırıldı.

Bulgular: Akut böbrek hasarı, tekrarlayan miyokart enfarktüsü, hedef damar revaskülarizasyonu, stent trombozu, kardiyojenik şok ve tüm nedenli ölüm sıklığı diğer gruplara kıyasla T3'te daha yüksekti. Uzun dönemdeki tüm nedenli ölümler de aynı şekilde diğer gruplara kıyasla T3'te daha yüksekti. Çoklu regresyon analizinde yüksek PCT düzeyi mortalite ile bağımsız olarak ilişkili bulundu.

Sonuç: Mevcut çalışmada STYME hastalarında yüksek PCT değerleri artmış hastane içi ve uzun dönem olumsuz olaylarla ilişkili bulundu.

Anahtar Kelimeler: Miyokart enfarktüsü, plateletkrit, mortalite

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Introduction

Platelets, like other hematopoietic cells, are produced in the bone marrow and are so small that they even miss a nucleus (1). Platelet activation plays a key role for coronary thrombosis and occlusion after coronary plaque rupture that is defined as Acute Coronary syndromes (ACS). ST segment elevation myocardial infarction (STEMI) is the most severe form of the ACS, which is characterized by an abrupt vessel occlusion. Myocardial ischemia and subsequent necrosis follow vessel occlusion if left untreated (2).

Platelet volume, which varies from 7 to 13 femtoliter in normal healthy individuals (3), is a marker of platelet activation and is measured using the mean platelet volume (MPV) (4,5). Platelet mass, namely plateletcrit (PCT), is the total platelet mass which is calculated by multiplying platelet count with MPV (PCT= platelet count × MPV/10^7) (6) and is reported routinely in the complete blood count report. Since larger platelets are enzymatically more active than the smaller ones (7), the association of MPV and platelet count with high thrombotic states like non-STEMI have already been studied (4). Besides, there are studies showing higher PCT and mortality association among STEMI patients (8,9). However, there is a lack of conclusive literature about the association between PCT and STEMI patients that undergone primary percutaneous coronary intervention (PPCI) yet. We aimed to investigate the association of high PCT and in-hospital and long-term outcomes of STEMI in a high-volume center.

Material and Methods

Study Population

A total of 2.838 consecutive patients with STEMI, who were admitted to a high-volume tertiary cardiovascular hospital (>2.500 PCIs/year) from January 2012 to February 2014, were retrospectively investigated. The study was approved by the local ethics committee (28.08.2020, 20/312), and due to the retrospective nature, there was no opportunity to obtain written informed consent from the patients. The data were anonymized by removing personally identifiable information.

STEMI diagnosis was made according to the following criteria: (1) typical chest pain lasting for >30 minutes and (2) ST-segment elevation in at least 2 contiguous leads with the following cutoff points: at least 0.2 mV in men or at least 0.15 mV in women in leads V2-V3 and/or at least 0.1 mV in the other leads or a definite/probable new left bundle branch block in electrocardiogram (ECG) later confirmed by creatine kinase (CK) and CK-myocardial band (CK-MB) isoenzyme increases and/or troponin increases (10).

We excluded patients (I) without appropriate STEMI diagnosis (N=105), (II) without PCT values at admission (N=73), (III) without data about previous medical treatment (N=56), (IV) with acute or chronic hepatic failure (N=33), (V) with chronic obstructive pulmonary disease (N=38), (VI) with peripheral and cerebral arterial disease (N=21), (VII) with inflammatory diseases, acute or chronic infectious disease (N=41), (VII) with autoimmune diseases, malignancies (N=9), (IX) with hypothyroidism, hyperthyroidism (N=11), (X) with cardiomyopathies and heart failure (N=17), and (XI) with severe valvular disease (N=19) (Figure 1). After the exclusion of the patients according the criteria above, a total of 2.415 patients were eligible to the study. The study population was divided into tertiles according to the PCT values [T1 (N=805), T2 (N=805), T3 (N=805)].

Data Collection

Demographic information and the clinical history of risk factors were obtained from medical data. Blood samples were obtained as soon as the STEMI diagnosis was made [in the emergency room (ER)]. Platelet count and MPV were measured by Coulter LH 780 Hematology Analyzer (Beckman Coulter Ireland Inc, Galway, Ireland). The PCTs were calculated according to the formula (PCT= platelet count × MPV/10,000). A 12-lead ECG was obtained for each patient as soon as the patent was seen in ER. Glomerular filtration rate (GFR) was estimated according to Cockcroft-Gault formula (11), and a transthoracic echocardiogram was performed using a system V (Vingmed; GE, Horten, Norway) with a 2.5-mHz phased array transducer. The left ventricular ejection fraction (LVEF) was measured with the modified Simpson's rule (12).

Coronary Angiography and PPCI

As soon as the diagnosis of STEMI was made, a loading dose of 300 mg of chewable acetylsalicylic acid and a P2Y12

Figure 1. Study flow chart
STEMI: Segment elevation myocardial infarction
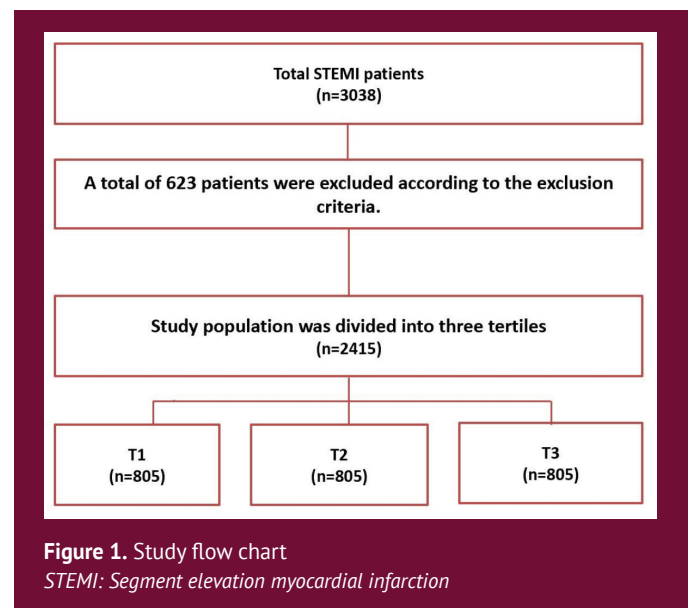
inhibitor were administered. All interventions were performed via the insertion of a 6F femoral sheath (Super Sheath; Boston Scientific, Natick, Massachusetts) to either the right or left common femoral artery. An intravenous bolus (100 IU/kg) of unfractionated heparin was administered through venous access in patients without enoxaparin administration previously. An activated clotting time of ≥200 seconds was aimed at prolonged interventions with more unfractionated heparin addition. Patients with initial administration of 1 mg/kg of enoxaparin within 8 hours of the intervention were followed without an additional enoxaparin booster, while 0.3 mg/kg of additional enoxaparin booster was administered to patients with 8 to 12 hours of enoxaparin administration. Predilation with a balloon angioplasty was performed whenever needed before stent deployment. Thrombus aspiration catheters were used whenever needed. The interventional cardiologists (ICs) decided the type of coronary stent according to the national and international guidelines and recommendations. Glycoprotein IIb/IIIa inhibitor usage was also up to the ICs preference. Intracoronary administration of adenosine, nitrates, and calcium channel blockers was also allowed according to the preference of the IC in cases of NR during PPCI.

Definitions

Hypertension (HT) was defined as having a blood pressure ≥140/90 mmHg at admission and/or being on anti-hypertensive drugs. Diabetes mellitus (DM) was defined as having (I) a random plasma glucose level ≥200 mg/dL, (II) a fasting plasma glucose level ≥126 mg/dL, (III) an HA1C ≥6.5% and/or being on antidiabetic drugs. Hyperlipidemia (HL) was defined as having a total cholesterol level ≥240 mg/dL, a serum triglyceride ≥200 mg/dL, low-density lipoprotein cholesterol ≥130 mg/dL and/or being on anti-hyperlipidemic drugs. Chronic kidney disease (CKD) was defined as having an eGFR <60 mL/min/1.73 m^2 and/or being on hemodialysis. Door-to-balloon time was the duration from admission to balloon inflation in the infarct related artery. The no-reflow phenomenon was defined as having a TIMI blood flow <3 after the PPCI. In-hospital mortality was defined as death from any cause during hospitalization. Cardiogenic shock was defined as systolic pressure <90 mmHg or systolic pressure drop greater than or equal to 40 mmHg for >15 min without new-onset arrhythmia, hypovolemia, or sepsis. Follow-up data were obtained from hospital records or by interviewing (directly or by telephone) patients, their families, or their personal physicians.

Statistical Analysis

The study population was divided into tertiles based on PCT values on admission. The Kolmogorov-Smirnov test was used for testing normality. Continuous variables with normal distributions were expressed as mean ± standard deviation (SD) and compared using One-Way analysis of variance. Continuous variables without normal distributions were expressed as mean ± SD and compared using the Kruskal-Wallis test. Categorical variables were expressed as number and percentages and the Pearson's χ^2 or Fisher's Exact tests were used to evaluate the differences. A backward stepwise multivariate Cox regression analysis, which included variables with p<0.1, was performed to identify independent predictors of cardiovascular mortality. The cumulative survival curve for cardiovascular mortality was constructed using the Kaplan-Meier method, with the differences assessed using the log-rank test. All statistical analyses were carried out using SPSS (version 20.0; IBM, Chicago, IL).

Results

Baseline characteristics and laboratory findings were showed in Table 1. T1 had a mean plateletcrit of 0.156±0.02% while the T2 had 0.209±0.01% and the T3 had 0.284±0.05%. The patients in T3 were older than the other tertiles (p= <0.001). There were more female in T3 than in the other tertiles (p= <0.001). HT (p=0.027), DM (p=0.008) and HL (p=0.020) were also more common in T3 than in the other tertiles. Left ventricle ejection fraction was lower in T3 than in the other tertiles (p= <0.001). The T3 had lower eGFR, higher admission glucose, higher peak CK-MB, higher white blood cell count than the other tertiles while the T2 had higher hematocrit levels than the others. There was no difference in relation of in-hospital medication.

Angiographic and procedural properties of the patients were presented in Table 2. There were no differences with regard to stent type (bare metal or drug eluting), intervention type (direct stenting, PTCA and stenting or only PTCA), number of vessels affected and TIMI blood flow after intervention.

In-hospital and long-term outcomes of the patients were presented in Table 3. Higher in-hospital MACE were observed in T3. Acute kidney injury (p=0.027), recurrent myocardial infarction (p=<0.001), target lesion revascularization (p=0.001), stent thrombosis (p=0.003), cardiogenic shock (p= <0.001) and all-cause mortality (p= <0.001) were altogether more common in T3 than the other tertiles. Out of hospital course was also unfavorable for T3. All-cause mortality in long term was higher in T3 than in the other tertiles (p= <0.001). The Kaplan-Meier cumulative survival curve was shown in Figure 2.

Univariate and multivariate analysis of risk factors for mortality was presented in Table 4. In univariate analysis, high plateletcrit [odds ratio (OR) =2.88, 95% confidence interval (CI): 2.08-3.98, p<0.001], age (OR =1.07, 95% CI: 1.06-1.08, p<0.001), DM (OR =1.83, 95% CI: 1.32-2.53, p<0.001), CKD (OR =1.91, 95% CI: 1.36-2.67, p<0.001), Killip score (OR =1.67, 95% CI: 1.15-

Table 1. Clinical and laboratory characteristics of patients with STEMI divided into 3 groups according to plateletcrit				
	T1 (n=805)	T2 (n=805)	T3 (n=805)	p
Baseline characteristics Age, y	57.5±11.5	57.6±11.9	59.8±12.1	**<0.001**
Body mass index, kg/m^2	27.2±3.6	27.4±3.8	27.6±3.8	0.103
Male gender	695 (86.3)	668 (83)	595 (73.9)	**<0.001**
Hypertension	283 (35.2)	298 (37)	334 (41.5)	**0.027**
Diabetes mellitus	232 (28.8)	257 (31.9)	290 (36)	**0.008**
Hyperlipidemia	241 (29.9)	255 (31.7)	292 (36.3)	**0.020**
Current smoking status	271 (33.7)	268 (33.3)	251 (31.2)	0.519
Myocardial infarction	158 (19.6)	161 (20)	148 (18.4)	0.691
Percutaneous coronary intervention	182 (22.6)	175 (21.7)	160 (19.9)	0.393
Coronary artery by-pass graft surgery	48 (6)	34 (4.2)	38 (4.7)	0.255
Chronic kidney disease	43 (5.3)	42 (5.29)	43 (5.3)	0.992
At admission Door-to-balloon time, minutes	19.8±9.4	20.4±10.2	19.7±9.4	0.352
Heart rate, beats per minute	76±15	75±15	75±16	0.279
Systolic blood pressure, mm Hg	135.9±24.3	135.4±23.9	135.1±25	0.782
Diastolic blood pressure, mm Hg	71.4±13.8	70.5±13.4	70.1±13.2	0.248
Killip classification	1.1.±0.4	1.1±0.4	1.1±0.5	0.134
Left ventricular ejection fraction	49.5±10.3	49.1±10.2	47.2±11.2	**<0.001**
Anterior myocardial infarction	348 (43.2)	372 (46.2)	385 (47.8)	0.171
Admission laboratory variables creatinine, mg/dL	0.95±0.4	0.91±0.3	0.94±0.5	0.079
GFR (Cockcroft-Gault), mL/min/1.73 m^2	104.4±36.8	109.5±38.1	106.3±38	**0.026**
Glucose, mg/dL	146.6±63.7	150.1±71	168±88.8	**<0.001**
Peak creatine kinase-MB, ng/mL	120.4±121.5	141.1±144.1	159.7±158.1	**<0.001**
White blood cell count, 10^3/μL	10.2±4.4	11.5±4.4	13.17±5.2	**<0.001**
Hematocrit, %	40.1±5.3	41.4±4.8	40.1±5.9	**<0.001**
Platelet count, 10^3/μL	180.2±36.4	239±27.6	320±74.8	**<0.001**
Mean platelet volume, fL	8.7±1.0	8.8±0.9	8.9±1.0	**<0.001**
Plateletcrit, %	0.156±0.02	0.209±0.01	0.284±0.05	**<0.001**
In-hospital medication Aspirin	789 (98)	791 (98.2)	783 (97.2)	0.479
PY212 receptor inhibitors	779 (96.7)	775 (96.2)	776 (96.3)	0.743
B-blocker	708 (87.9)	711 (88.3)	706 (87.7)	0.893
Calcium channel blocker	127 (15.7)	133 (16.5)	131 (16.2)	0.698
Statin	692 (86)	699 (86.8)	701 (87)	0.790
Diuretics	86 (10.6)	80 (9.9)	88 (11)	0.643
ACEI or ARB	721 (89.5)	715 (88.8)	718 (89.1)	0.842
Insulin	164 (20.3)	178 (22.1)	173 (21.4)	0.543
GFR: Glomerular filtration rate, ACEI: Angiotensin converting enzyme inhibitor, ARB: Angiotensin receptor blocker, STEMI: Segment elevation myocardial infarction				

2.38, p=0.002), LVEF (OR =0.95, 95% CI: 0.93-0.97, p<0.001), peak CK-MB (OR =1.00, 95% CI: 1.00-1.00, p<0.001), and white blood cell count (OR =1.29, 95% CI: 1.09-1.62, p=0.002) were found to be the predictors of mortality.

In the multivariable regression analysis, using a model adjusted for the aforementioned parameters, high plateletcrit (OR =2.41, 95% CI: 2.12-2.70, p<0.001), age (OR =1.06, 95% CI: 1.05-1.07, p<0.001), DM (OR =1.54, 95% CI: 0.98-2.16, p=0.013),

CKD (OR =1.78, 95% CI: 1.14-2.44, p=0.002), Killip score (OR =1.42, 95% CI: 1.06-2.81, p=0.025), LVEF (OR =0.93, 95% CI: 0.91-0.95, p<0.001), and peak CK-MB (OR =1.00, 95% CI: 1.00-1.00, p<0.003) were found to be independent predictors for mortality.

Discussion

The study showed higher in-hospital and long-term mortality among the patients with higher PCT values in STEMI. PCT was found to be an independent predictor for mortality in this

Figure 2. Kaplan-Meier survival curve
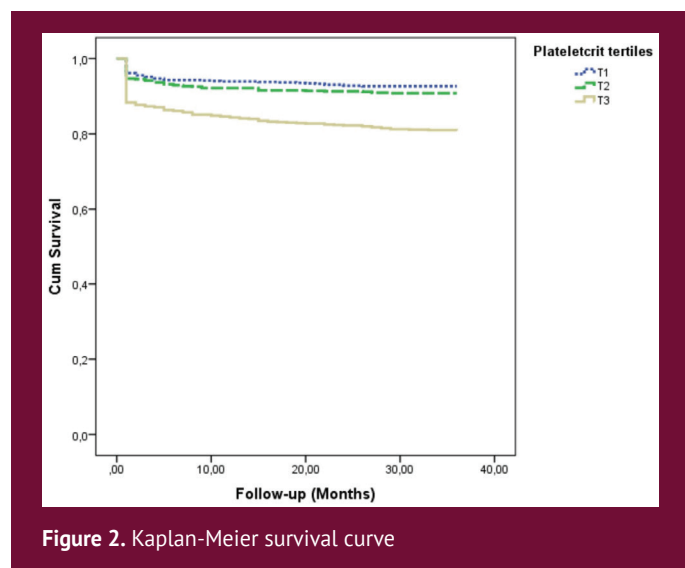
setting besides age, DM, CKD, Killip score, left ventricle ejection fraction and peak CK-MB.

Larger platelets are biologically more active and powerful than the smaller one with regard to pro-thrombotic properties. MPV was found to show a high specificity and predictive value for coronary slow flow, stable ischemic heart diseases, and ACSs (13,14,15). Both MPV and PCT were found to be higher in saphenous vein graft disease after by-pass surgery (16) and were found to be associated with short-term outcomes of ischemic stroke (17). Moreover, PCT was related to increased venous thromboembolism risk in females (18). Impact of PCT was studied in high thrombus burden clinical scenario in two separate studies (8,9). Cetin et al. (9) observed higher PDW and PCT values in young STEMI patients than in the older ones, which is reasoned with platelet activity in the young ones. On the other hand, Ugur et al. (8) showed increased long-term mortality in STEMI patients with higher PCT values. Despite the fact that patients with higher PCT showed more in-hospital shock, inotrope use and lower ejection fraction, there were no statistically significant difference in relation of in-hospital mortality (8). We showed increased acute kidney injury, target lesion revascularization, stent thrombosis, cardiogenic shock and in-hospital mortality among the patients with higher PCT values besides increased long term mortality.

The fourth universal definition of MI guideline defines type 1 MI as having an occlusive or non-occlusive intravascular thrombosis secondary to atherosclerotic plaque disruption

Table 2. Angiographic analysis of patients with STEMI divided into 3 groups according to plateletcrit				
	T1 (n=805)	T2 (n=805)	T3 (n=805)	p
Vessel stenosis (>50%) 1 vessel	446 (55.4)	423 (52.5)	436 (54.1)	0.327
2 vessels	208 (25.8)	222 (27.6)	223 (27.7)	0.523
3 vessels	151 (18.8)	160 (19.9)	146 (18.1)	0.751
Intervention type Direct stenting	31 (3.8)	33 (4)	31 (3.8)	0.971
PTCA and stenting	651 (80.8)	644 (80)	655 (81.39)	0.893
Only PTCA	123 (15.2)	128 (15.9)	119 (14.7)	0.815
Stent type Drug eluting stent	587 (87.3)	577 (85.2)	589 (85.8)	0.279
Bare metal stent	85 (12.6)	100 (14.7)	97 (14.1)	0.311
TIMI blood flow after intervention TIMI 0	38 (4.7)	54 (6.7)	50 (6.2)	0.161
TIMI1	13 (1.6)	12 (1.5)	23 (2.9)	0.235
TIMI 2	44 (5.5)	39 (4.8)	49 (6.1)	0.125
TIMI 3	710 (88.2)	700 (87)	683 (85.8)	0.269
No-reflow phenomenon	95 (11.8)	105 (13)	122 (15.2)	0.135
PTCA: percutaneous transluminal coronary angioplasty, TIMI: Thrombolysis in myocardial infarction				

(rupture or erosion) (19). An increased pro-inflammatory and pro-thrombotic activity with unbalanced stress may cause a plaque rupture, subsequent thrombosis formation, and complete occlusion. A vicious circle between increased thrombosis and inflammation is further aggravated with increased release of pro-inflammatory cytokines. Megakaryocyte proliferation is promoted with interleukin (IL)-1, IL-3, and IL-6 that increases circulating platelet count with larger and reactive ones (20,21). Increased platelet mass (PCT) may show higher pro-thrombotic and pro-inflammatory activity which could be associated with worse outcomes in STEMI setting. It is already showed that patients with heavy thrombus burden suffer more in-hospital and long-term mortality than the others in STEMI (22).

Study Limitations

This study had several limitations. It is a single-center, retrospective study without randomization and thus subject to selection bias; however, consecutive patients were selected to lessen possible effects of this limitation. In addition, inflammatory markers such as high-sensitive C-reactive protein, B-type natriuretic peptide, other proinflammatory cytokines, and markers of oxidative stress were not analyzed. Despite adjusting for multiple risk factors, it is possible that there might have been residual confounding conditions and medications.

Conclusion

This is the first study to show high in-hospital and long-term mortality of STEMI patients with high PCT values at admission.

Table 3. In-hospital and long-term outcomes of patients with STEMI divided into 3 groups according to plateletcrit levels				
	T1	T2	T3	p
In-hospital outcomes Acute kidney injury	89 (11.1)	96 (11.9)	123 (15.3)	0.027
Recurrent myocardial infarction	17 (2.1)	29 (3.6)	62 (7.7)	<0.001
Target lesion revascularization	28 (3.5)	38 (4.7)	60 (7.5)	0.001
Stent thrombosis	15 (1.9)	28 (3.5)	40 (5.0)	0.003
Cardiogenic shock	26 (3.2)	35 (4.3)	65 (8.1)	<0.001
All-cause mortality	30 (3.7)	39 (4.8)	92 (11.4)	<0.001
Out-hospital course Follow-up time (months)	33.7±8.2	33.1±9.1	30.2±12.4	<0.001
All-cause mortality	29 (3.7)	35 (4.6)	62 (8.7)	<0.001

Table 4. Univariate analysis and multivariate model for in-hospital mortality					
Univariate analysis	p	OR (95% CI)	Multivariate analysis	p	OR (95% CI)
High plateletcrit	<0.001	2.88 (2.08-3.98)	High plateletcrit	<0.001	2.41 (2.12-2.70)
Age	<0.001	1.07 (1.06-1.08)	Age	<0.001	1.06 (1.05-1.07)
Male gender	0.077	1.40 (0.96-2.04)			
Body mass index	0.274	0.97 (0.93-1.01)			
Hypertension	0.065	1.35 (0.98-1.86)			
Diabetes mellitus	<0.001	1.83 (1.32-2.53)	Diabetes Mellitus	0.013	1.54 (0.98-2.16)
Hyperlipidemia	0.015	1.50 (1.08-2.08)			
Smoking	0.685	1.07 (0.76-1.50)			
Chronic kidney disease	<0.001	1.91 (1.36-2.67)	Chronic kidney disease	0.002	1.78 (1.14-2.44)
Previous myocardial infarction	0.004	1.64 (1.14-2.45)			
Killip score	0.002	1.67 (1.15-2.38)	Killip score	0.025	1.42 (1.06-2.81)
Anterior myocardial infarction	0.008	1.60 (1.12-2.67)			
Left ventricular ejection fraction	<0.001	0.95 (0.93-0.97)	Left ventricular ejection fraction	<0.001	0.93 (0.91-0.95)
Peak creatine kinase-MB	<0.001	1.00 (1.00-1.00)	Peak creatine kinase-MB	0.003	1.00 (1.00-1.00)
White blood cell count	0.002	1.29 (1.09-1.62)			
Hematocrit	0.308	0.98 (0.82-1.16)			
OR: Odds ratio, CI: Confidence interval, MB: Myocardial band					

High PCT was found as an independent risk factor for both in-hospital and long-term cardiovascular mortality age, DM, CKD, Killip score, left ventricle ejection fraction and peak CK-MB.

Ethics

Ethics Committee Approval: The study was approved by the local ethics committee (28.08.2020, 20/312).

Informed Consent: Due to the retrospective nature, there was no opportunity to obtain written informed consent from the patients.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: M.K., A.K., G.İ., O.B., S.D., Concept: M.K., A.K., G.İ., O.B., S.D., Design: M.K., A.K., G.İ., O.B., S.D., F.Ö., A.L.O., Data Collection or Processing: M.K., A.K., G.İ., O.B., S.D., Analysis or Interpretation: M.K., A.K., G.İ., O.B., S.D., Literature Search: M.K., A.K., G.İ., O.B., S.D., F.Ö., Writing: M.K., A.K., G.İ.

Conflict of Interest: No conflict of interest was declared by the authors.

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Neutrophil-to-Lymphocyte Ratio for the Assessment of Long-term Mortality in Patients with Heart Failure

Kalp Yetersizliği Hastalarında Nötrofil-lenfosit Oranının Uzun Dönem Mortalite ile İlişkisi

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ABSTRACT

Background: The neutrophil-to-lymphocyte ratio (NLR) is a novel inflammatory parameter, which has a predictor value in different cardiovascular disorders. The objective of this study is to evaluate the predictive value of the NLR for long-term mortality (5-year) in patients with heart failure.

Materials and Methods: We retrospectively investigated the association between NLR and mortality in 785 patients who presented with CHF between 2006 and 2010. Of these, 201 patients were excluded because of factors that could impact the NLR level. Five hundred eighty four patients were categorized by the respect of low (NLR <1.5), intermediate (1.5≤ NLR <3), and high (NLR ≥3) tertiles of NLR and were followed for 5 years. Cox-proportional regression analysis was used to establish the relationship between NLR levels and all-cause long-term mortality.

Results: The mean NLR was 2.82±2.54. The lowest long-term mortality was determined in patients with NLR level of <1.5. In a multivariable Cox-proportional regression analysis, the mortality risk was higher for patients with NLR of ≥1.5 [hazard ratio (HR), 1.98, 95% confidence interval (CI) 0.98-3.42 and HR, 4.22, 95% CI 2.66-5.86, for patients with 1.5≤ NLR <3 and NLR ≥3, respectively]. Additionally, NLR showed a significant correlation with B-type natriuretic peptide (BNP). The Spearman correlation coefficient identified the significant threshold effect (coefficient =0.34, p<0.001).

Conclusion: We detected a significant association between the NLR and long-term mortality. Additionall, NLR showed a significant correlation with BNP. Therefore, NLR may become a simple and cheap biomarker in the state of unavailability of BNP.

Keywords: Neutrophil-to-lymphocyte ratio, heart failure, mortality

ÖZ

Amaç: Nötrofil-lenfosit oranı (NLO) yeni bir enflamatuvar parametre olup farklı kardiyovasküler alanlarda prediktif öneme sahiptir. Bu çalışmanın amacı NLO'nun kalp yetersizliği hastalarının 5-yıllık sağkalımındaki rolünü araştırmaktır.

Gereç ve Yöntemler: Bu retrospektif çalışmada 2006-2010 yılları arasında hastanemizde yatan 785 kalp yetersizliği hastası araştırılmıştır. NLO seviyesini etkileyebilecek durumu olan 201 hasta çalışmadan çıkarıldı ve kalan 584 hasta NLO seviyesine göre 3 gruba ayrıldı; düşük (NLO <1,5), orta (1,5≤ NLR<3) ve yüksek (NLR ≥3) ve bu hastaların 5 yıllık sağkalımları kayıt edildi. NLO'nun uzun dönemdeki bağımsız ilişkisini araştırmak için Cox-regresyon analizi kullanıldı.

Bulgular: Ortalama NLO değeri 2,82±2,54 olarak bulundu. En düşük mortalite NLO <1,5 olan hastalarda izlendi. Çoklu değişkenli Cox-regresyon analizinde mortalite riski NLO ≥1,5 olan hastalarda daha fazla bulundu [sırasıyla 1,5≤ NLO <3 ve NLO ≥3 olanlar için risk oranı (RO), 1,98, %95 güven aralığı (GA) 0,98-3,42 ve RO, 4,22, %95 GA 2,66-5,86]. Ayrıca NLO ile BNP arasında ciddi bir korelasyon saptandı (Spearman korelasyon katsayısı: 0,34, p<0,001).

Sonuç: Çalışmamızda NLO ile uzun dönem mortalite arasında bağımsız bir ilişki saptandı. Ayrıca NLO ile BNP arasında ciddi bir korelasyon izlendi. BNP'ye ulaşılamadığı durumlarda NLO ucuz ve basit bir biyobelirteç olarak kullanılabilir.

Anahtar Kelimeler: Nötrofil-lenfosit oranı, kalp yetersizliği, mortalite

Introduction

Despite the fact that in medical and device-dependent treatments, physicians from all departments still encounter congestive heart failure (CHF) frequently in the clinical practice as a common health and heart problem with high morbidity and mortality. There are current studies showing that inflammation has a substantial predictive role in the initiation and progression of cardiovascular diseases (1,2). Brain-type natriuretic peptide (BNP) is commonly suggested as an exclusion criterion in CHF. Furthermore, BNP is a great prognostic value in patients with CHF (3). However, BNP is relatively expensive and is not available in all health centers. Complete blood count test is a cheap and widespread test which is in use. Usage of hematological parameters as an prognostic and predictive marker in cardiovascular diseases has been intensively investigated in recent years (4,5,6). Among the different hematological parameters, neutrophil-to-lymphocyte (NLR) has become prominent as a marker of underlying inflammation. The aim of the current study is to evaluate the predictive value of NLR in long-term mortality. Additionally, we have hypothesized that NLR may become a diagnostic parameter in case of unavailability of BNP.

Material and Methods

Between January 2006 and December 2010, 786 consecutive patients diagnosed with CHF, who were admitted to our hospital, were evaluated retrospectively for the current study. CHF is defined as a syndrome in which patients have typical symptoms (e.g. breathlessness, ankle swelling, and fatigue) and signs (e.g. elevated jugular venous pressure, pulmonary crackles, and displaced apex beat) resulting from an abnormality of cardiac structure or function (3). The current study was based on the patients with chronic CHF. A total of 201 patients who had an affecting factor that could change the NLR levels, including systemic disease and use of medical treatment with chemotherapy, having evidence of any concomitant inflammatory disorder, acute and chronic infection, receiving the glucocorticoid therapy within the past 3 months, having Acute Coronary syndrome and percutaneous coronary intervention in the past 6 months, having secondary hypertension and acute decompensated CHF, were excluded. This study was approved by the institutional ethical and scientific committee.

Data Sources

Clinical, demographic, historical, angiographic, treatment and laboratory data were obtained from the hospital's medical database. Complete blood counts, which included total white blood cell, neutrophils, lymphocytes, monocytes, and eosinophils, were obtained at the time of admission. All patients were evaluated for the presence of cardiovascular risk factors such as hypertension, hyperlipidemia, diabetes mellitus, and smoking status. Echocardiogram was performed by trained cardiologists and left ventricular ejection fraction (LVEF) was calculated by using the Simpson method (7). Left ventricular dilatation is defined as a left ventricular diastolic diameter of >60 mm. The estimated glomerular filtration rate (eGFR) was calculated by using the Cockcroft-Gault equation (8). The drugs were administered during the hospitalization according to the European Society of Cardiology Guidelines (3). NLR was calculated as the ratio of neutrophil count to lymphocyte count. NLR levels for each patient were collected and reviewed by a trained coordinator. Patients were classified into 3 groups to evaluate the association between the long-term mortality and NLR levels: ≤1.5, 1.5 to 3.0, and ≥3.0. The aim of this study was to evaluate the prognostic impact of the NLR on long-term mortality in patients with CHF. The evaluation of mortality was obtained from hospital's medical database or by follow-up interviews (directly or by telephone). The primary end point was long-term (5-year) mortality

Statistical Analysis

Baseline characteristics were compared among the patients by NLR level and categorized accordingly as ≤1.5, 1.5 to <3.0, and ≥3.0. The Kolmogorov-Smirnov test was used for evaluating normality. Continuous variables with normal distributions were expressed as mean ± standard deviation and compared using One-Way analysis of variance. Continuous variables with skewed distributions were expressed as median (25th and 75th percentiles) and compared using the Kruskal-Wallis test. Categorical variables were expressed as number and percentages and the Pearson's chi-square or Fisher's Exact tests were used to evaluate the differences. Correlation analyses between the NLR and BNP were made using the Spearman test. The receiver operating characteristic (ROC) analysis was used to assess the ability of the NLR and BNP to predict the mortality. After follow-up periods of 24.32±15.81 months, the median survival time of three groups was compared using the Kaplan-Meier survival method. Overall survival (OS) was calculated from the day of diagnosis to the day of death or last follow-up. Patients lost to follow-up were censored at the time of last follow-up. Differences between the groups were analyzed by the log-rank test. A forward Cox-proportional regression model was used for multivariable analysis. The hazard ratios indicate the relative risk of death in NLR level subgroup compared to those in the lowest-risk subgroup (NLR <1.5). In multivariable models, confounders in bivariate analysis as predictors of long-term mortality were considered. Five different models were generated to obtain the impact of potential confounders on the association between NLR level and mortality. These 5 models include: (1) unadjusted; (2) adjusted for age, sex; (3) adjusted

for comorbidities and eGFR; (3) adjusted for treatments; (5) adjusted for all confounders including demographics (age, gender), first measurement of the following laboratory values (creatinine, brain type natriuretic peptide, admission glomerular filtration rate calculated by Cockcroft Gault, glucose, sodium, potassium, C-reactive protein, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, gama-glutamyl transferase, total bilirubin, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, hematocrit, red cell distribution width, platelet count, mean platelet volume, plateletcrit), comorbidities (diabetes, hypertension, hyperlipidemia, cerebrovascular disease, chronic obstructive pulmonary disease, atrial fibrillation, left ventricular dilatation), and treatments. Acute kidney injury is defined as an increase in serum creatinine level of ≥0.3 mg/dL or a relative increase in serum creatinine level of ≥50% (9,10). A two-tailed p-value of <0.05 was considered as statistically significant, and 95% CIs were presented for hazard ratios. Analyses were performed using the Statistical Package for Social Sciences software, version 20.0 (SPSS; IBM, Armonk, New York, USA).

Results

A total of 584 patients (mean age 42.6±10.9 years; men 81%) with CHF were included. There was a significant difference in terms of gender age (p<0.001) among the subgroups of NLR whereas there was no significant difference in terms of gender (p=0.076). The patients' baseline characteristics, categorized by NLR level, are listed in Table 1. Overall, NLR was significantly correlated directly with BNP (r=0.34, p<0.001). The groups were similar in terms of history of hypertension, diabetes mellitus, current smoking status, ischemic heart failure, percutaneous coronary intervention, coronary artery bypass graft surgery, and atrial fibrillation. Hyperlipidemia was more common in patients with NLR of <1.5, whereas chronic kidney disease was more common in patient with NLR of ≥3. At admission, there were significant differences in terms of systolic blood pressure (0.012) and diastolic blood pressure (0.008). The New York Heart Association status was higher and LVEF was lower in patients with INR level of ≥3. In admission laboratory variables; BNP, blood fasting glucose, creatine, C-reactive protein, aspartate aminotransferase, alanine aminotransferase, gama glutamyl transferase and total bilirubin were higher in patients with serum NLR level of ≥3. Among the hematological parameters; white blood cell count, netrophil count, monocyte count, eosinophil count and red cell distribution width were higher in patients with serum NLR level of ≥3. In treatments; inverter cardioverter defibrillator or cardiac resynchronization therapy, digoxine, mineralocorticoid receptor antagonist and furosemide were more commonly used in patients with NLR level of ≥

3. A correlation analysis performed using a Spearman test revealed that there was a correlation between the NLR and the BNP in the study population (r=0.340, p<0.001) (Figure 1). After ROC analysis, the best cut-off value of the BNP to predict the mortality was 445 pg/mL with 71% sensitivity and 80% specificity [area under the curve (AUC): 0.83; 95% CI: 0.78-0.87, p<0.001], and the best cut-off value of the NLR to predict the mortality was 2.3 with 71% sensitivity and 63% specificity (AUC: 0.74, 95% CI: 0.68-0.78, p<0.001) (Figure 2).

The patients were followed up for a mean period of 24.32±15.81 months. The 5-year Kaplan-Meier OS for NLR level of <1.5, 1.5 to 3.0 and >3.0 were 91.2%, 77.6% and 53.7%, respectively. The Kaplan-Meier cumulative survival curve was shown in Figure 3. Table 2 lists unadjusted and adjusted Cox-proportional regression analysis for long-mortality categorized by NLR levels. The long-term mortality had the highest rates at NLR level of >3 and those were 4.5-times higher mortality rates (95% CI: 2.35-8.78) than NLR level of <1.5, which had the lowest rates and which was used as the reference. This relationship persisted even after the adjustment for all confounders. Patients with NLR level of 1.5 to 3 was also had 2-times higher mortality rates (95% CI: 0.97-3.67) than the reference group.

Discussion

Inflammatory response has commonly been considered in both the progression and clinical worsening of HF. In the current study, we evaluated the leukocytic response in heart failure and its possible relationship with long-term mortality. The main findings of the current study were that a high NLR at hospital admission was associated with increased long-term mortality
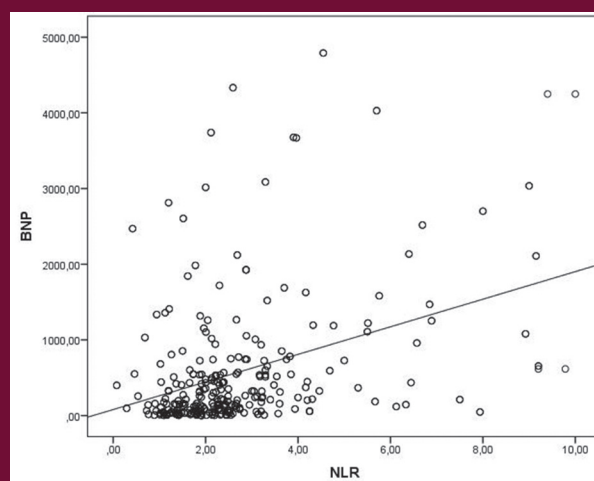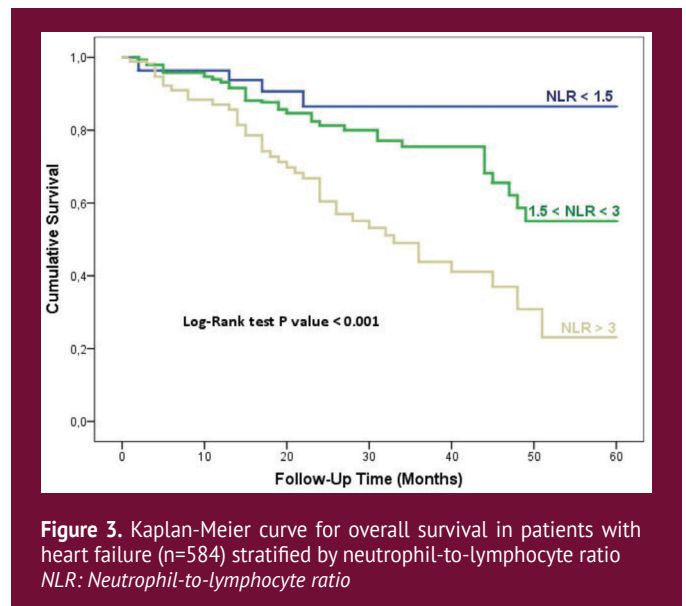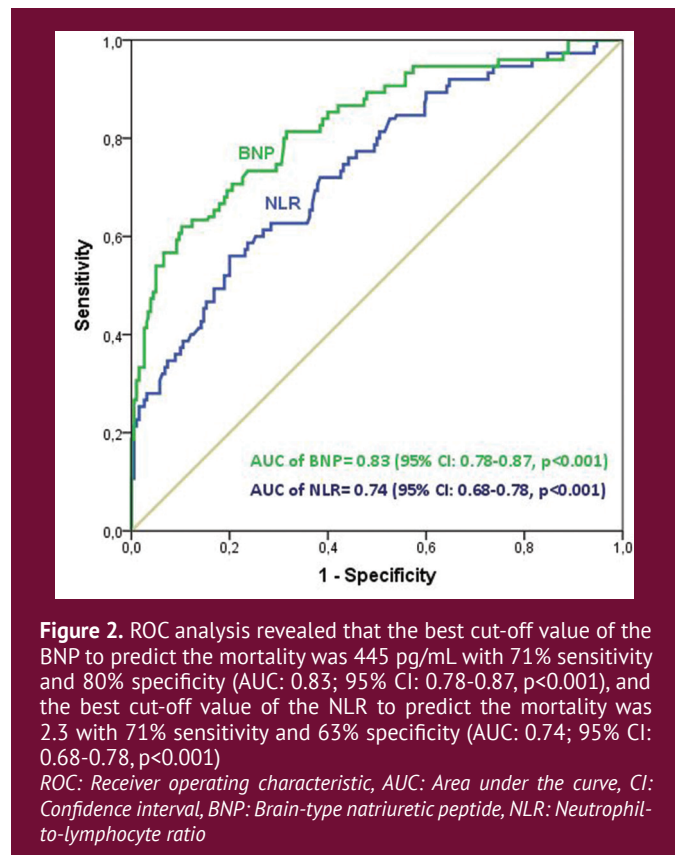

Figure 1. Correlation analysis of levels of neutrophil-to-lymphocyte ratio and levels of brain-type natriuretic peptide. Correlation analysis was performed by the Spearman rank correlation test (r= 0.34, p<0.001)
BNP: Brain-type natriuretic peptide, NLR: Neutrophil-to-lymphocyte ratio

Table 1. Baseline characteristics of patients classified by neutrophil-to- lymphocyte ratio				
	Neutrophil- to- lymphocyte ratio (n=584)			
	<1.5 (n=114)	1.5 to 3 (n=294)	>3 (n=175)	p
Age	41±11	42±10	45±12	<0.001
Male, gender	98 (86.0)	228 (77.6)	147 (84.0)	0.076
Body mass index	28.8±5.4	27.3±5.3	26.8±4.8	0.007
Neutrophil- to- lymphocyte ratio	1.1±0.3	2.1±0.3	4.8±2.9	<0.001
History Hypertension	38 (33.3)	72 (24.5)	52 (29.7)	0.162
Diabetes mellitus	18 (15.8)	34 (11.6)	31 (17.7)	0.159
Hyperlipidemia	46 (41.8)	108 (37.0)	44 (26.3)	0.016
Current smoking status	12 (10.5)	18 (6.1)	14 (8.0)	0.308
Ischemic heart failure	24 (21.1)	74 (25.2)	53 (30.3)	0.199
Percutaneous coronary intervention	10 (8.8)	20 (6.8)	17 (9.7)	0.509
Coronary artery bypass graft surgery	12 (10.5)	10 (3.4)	16 (9.1)	0.008
Chronic kidney disease	4 (3.5)	6 (2.0)	19 (10.9)	<0.001
Atrial fibrillation	10 (8.8)	28 (9.5)	22 (12.6)	0.482
At admission Systolic blood pressure, mm Hg	123±19	119±19	116±23	0.012
Diastolic blood pressure, mm Hg	79±13	76±13	74±13	0.008
Heart rate (beats per minute)	86±11	86±15	88±16	0.466
New York heart association status	2.1±0.4	2.0±0.5	2.4±0.8	<0.001
Left ventricular ejection fraction, %	35±47	25±8	23±8	<0.001
Left ventricular dilatation	92 (80.7)	222 (75.5)	132 (75.4)	0.499
Admission laboratory variables Brain type natriuretic peptide, pg/mL	334±573	453±694	932±1.098	<0.001
Creatinine (mg/dL)	0.9±0.2	0.9±0.2	1.0±0.3	<0.001
Estimated glomerular filtration rate, (Cockcroft-Gault), mL/min/1.73 m^2	124±36	115±33	74±13	<0.001
Glucose, mg/dL	105±42	105±35	112±40	0.002
Sodium, mEq/L	140±3	139±3	137±5	<0.001
Potassium, mEq/L	4.5±0.3	4.5±0.4	4.5±0.5	0.652
C-reactive protein	0.9±1.7	3.2±8.5	6.5±11.0	<0.001
Total protein, mg/dL	7.3±0.6	7.4±0.5	7.2±0.8	0.241
Albumin, mg/dL	4.3±0.5	4.4±0.3	4.3±0.5	0.001
Aspartate aminotransferase, IU/L	29±12	32±38	58±125	0.003
Alanine aminotransferase, IU/L	31±15	33±40	63±146	0.002
Gama glutamyl transferase, IU/L	58±45	77±144	96±104	0.001
Total bilirubin, mg/dL	0.8±0.7	0.8±0.5	1.3±1.1	<0.001
Admission blood count variables White blood cell count, cells/µL	7.9±2.0	8.3±1.7	9.0±2.3	<0.001
Neutrophil count, cells/µL	3.6±1.1	5.6±1.1	6.6±2.0	<0.001
Lymphocyte count, cells/µL	3.6±1.7	2.3±0.5	1.5±0.4	<0.001

Table 1. Continued	Neutrophil- to- lymphocyte ratio (n=584)			
	<1.5 (n=114)	1.5 to 3 (n=294)	>3 (n=175)	p
Monocyte count, cells/μL	0.5±0.7	0.6±0.4	0.7±0.8	<0.001
Eosinophil count, cells/μL	0.15±0.14	0.22±0.16	0.21±0.19	<0.001
Hematocrit, %	43.9±5.9	42.4±4.4	40.0±5.9	<0.001
Red cell distribution width	13.9±2.3	14.5±2.9	16.3±3.3	<0.001
Platelet count, cells/μL	257±70	240±72	262 ± 96	0.016
Plateletcrit, ng/mL	0.23±0.05	0.21±0.05	0.23 ± 0.08	0.147
Mean platelet volume, fL	9.1±1.0	9.2±1.3	9.1 ± 1.2	0.286
Treatments Aspirin	60 (52.6)	124 (42.2)	81 (46.8)	0.152
Inverter cardioverter defibrillator or cardiac resynchronization therapy	6 (5.3)	20 (6.9)	37 (21.9)	<0.001
Angiotensin-converting enzyme inhibitor	90 (78.9)	206 (70.1)	143 (81.7)	0.011
Angiotensin receptor blocker	20 (17.5)	52 (17.7)	24 (13.7)	0.502
B-blocker	114 (100.0)	290 (98.6)	161 (92.0)	<0.001
Digoxin	54 (47.4)	160 (54.4)	111 (63.4)	0.022
Mineralocorticoid receptor antagonist	96 (84.2)	250 (85.0)	163 (93.1)	0.021
Furosemide	88 (77.2)	216 (73.5)	159 (90.9)	<0.001
Continuous variables are presented as mean ± SD, nominal variables presented as frequency (%), SD: Standard deviation				

Figure 2. ROC analysis revealed that the best cut-off value of the BNP to predict the mortality was 445 pg/mL with 71% sensitivity and 80% specificity (AUC: 0.83; 95% CI: 0.78-0.87, p<0.001), and the best cut-off value of the NLR to predict the mortality was 2.3 with 71% sensitivity and 63% specificity (AUC: 0.74; 95% CI: 0.68-0.78, p<0.001)
ROC: Receiver operating characteristic, AUC: Area under the curve, CI: Confidence interval, BNP: Brain-type natriuretic peptide, NLR: Neutrophil-to-lymphocyte ratio

Figure 3. Kaplan-Meier curve for overall survival in patients with heart failure (n=584) stratified by neutrophil-to-lymphocyte ratio
NLR: Neutrophil-to-lymphocyte ratio

in patients with HF and the high NLR was strongly correlated with the BNP.

Despite the fact that many advances have been made in HF by the respect of diagnosis and treatment, it still carries a high morbidity and mortality. There are many studies of NLR that have been performed recently in all departments of medical sciences. NLR has been studied in cardiac diseases including acute coronary syndromes, infective endocarditis, and diastolic and systolic heart failure (6,11,12,13). Previous studies suggested significant predictive and prognostic value of NLR. Independently, either neutrophilia or lymphopenia has been related to increased morbidity and mortality in heart failure (14,15). The usefulness of NLR in patients with acute heart failure has been reported in an extensive review, Uthamalingam et al. (16) revealed a 26-month follow-up study of 1.212 patients with acute decompensated heart failure (16). This study presented that patients with high NLR had significantly higher in-hospital and long-term mortality. In another study, Benites-Zapara et al. (4) confirmed this finding. In their study; they examined the patients with advanced heart failure and revealed that patients with

high NLR had higher cardiac transplantation and mortality rates. Additionally, Durmus et al. (5) showed a significant relationship between increasing NLR and decreasing LVEF. All these studies suggest that inflammatory mechanisms have an important role in heart failure. Although these studies introduced a significant inverse correlation between NLR and heart failure, the patients with stable heart failure without acute decompensation were not examined in Uthamalingam et al.'s (16) study. Acute decompensating heart failure has a substational role in NLR, and these patients are more prone to death. Furthermore, Benites-Zapata et al. (4) included only the patients with advanced heart failure. Therefore, the patients with lower NYHA status were not evaluated in their study. The current study included all the consecutive patients with heart failure who were not in acute decompensating state according to the "ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012". Albeit the study population was different from the previous studies on heart failure, the results were similar. The long-term mortality had the highest rates at NLR level of >3 and that had 4.5-times higher mortality rates than NLR level of <1.5.

Another important issue in HF is diagnostic difficulty. Because the signs and symptoms of HF are non-specific, many patients with suspected HF referred for echocardiography are not found to have an important cardiac abnormality. Because of unavailability of echocardiography, an alternative approach to diagnosis is to measure the BNP (3,17,18). Commonly, a normal BNP level excludes significant cardiac disease. Despite the fact that BNP has a substantial role in the diagnosis of HF, it is still commonly not available in many hospitals especially in developing countries. This difficulty attenuates the diagnostic value of the natriuretic peptides. In the current study, NLR level showed a very strong correlation with the BNP (r=0.340, p<0.001). Furthermore, a cut off NLR value of 2.3 has 71% sensitivity and 63% specificity in predicting five-year long-term mortality. These findings suggest that NLR may become a simple and cheap biomarker in the diagnosis and prognosis of heart failure. Because the blood count is usually ordered in patients with HF, the ability to classify a HF population without additional testing is important.

Table 2. Cox-proportional analysis and 5-year mortality by neutrophil-to- lymphocyte ratio			
	Neutrophil to lymphocyte ratio (n= 584)		
	<1.5 (n=114)	1.5 to 3 (n=294)	>3 (n=175)
5-year mortality Number of deaths	10	66	81
Mortality, %	8.8	22.4	46.3
Mortality, HR (95% CI) Model 1: unadjusted	1 (reference)	1.90 (0.97-3.67)	4.55 (2.35-8.78)
Model 2: adjusted for age and gender	1 (reference)	1.93 (0.98-3.78)	4.68 (2.41-9.11)
Model 3: adjusted for comorbidities and GFR	1 (reference)	1.91 (0.92-3.79)	4.04 (2.53-6.02)
Model 4: adjusted for treatment	1 (reference)	1.96 (0.95-3.63)	4.97 (3.03-7.75)
Model 5: adjusted for all covariables[b]	1 (reference)	1.98 (0.98-3.42)	4.22 (2.66-5.86)

Abbreviations: GFR, glomerular filtration rate, HR, hazard ratio, [b]: Includes demographics (age, gender); first measurement of the following laboratory values (creatinine, brain type natriuretic peptide, admission glomerular filtration rate calculated by Cockcroft Gault, glucose, sodium, potassium, C-reactive protein, total protein, albumin, aspartate aminotransferase, alanine aminotransferase, gama-glutamyl transferase, total bilirubin, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, hematocrit, red cell distribution width, platelet count, mean platelet volume, plateletcrit); comorbidities (diabetes, hypertension, hyperlipidemia, cerebrovascular disease, chronic obstructive pulmonary disease, atrial fibrillation, left ventricular dilatation) and treatments. CI: Confidence interval, HR: Hazard ratio, GFR: Glomerular filtration rate

Study Limitations

The current study has several limitations. Firstly, this was a single center, retrospective and observational study. Secondly, the patients who underwent cardiac transplantation were not included. Thirdly, only baseline measures of NLR were evaluated. NLR values overtime and their effect on clinical outcomes were not obtained. Fourthly, due to the problems with insurance policies and hospital protocols, some of the patients had not inverter cardioverter defibrillator even if they were suitable.

Fifthly, although we used multivariable analysis, we could not exclude the possibility of residual unmeasured covariables which might influence the outcomes.

Conclusion

NLR is a non-invasive, widely available and cheap parameter for predicting long-term outcome in patients with HF. The current study results showed that patients with a high NLR had higher long-term mortality. Furthermore, because of significant correlation with BNP, NLR may be used as a diagnostic test for HF.

Ethics

Ethics Committee Approval: This study was approved by the institutional ethical and scientific committee.

Informed Consent: Consent was obtained from all patients or their relatives in our study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ü.K., N.G., K.K., Concept: Ü.K., N.G., K.K., Design: Ü.K., N.G., K.K., Data Collection or Processing: Ü.K., N.G., K.K., Analysis or Interpretation: Ü.K., N.G., K.K., Literature Search: Ü.K., N.G., K.K., Writing: Ü.K., N.G., K.K.

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Nodular Fasciitis Mimicking a Maxillofacial Cancer

Maksillofasiyal Kanseri Taklit Eden Nodüler Fasiit

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ABSTRACT

Nodular fasciitis is a rare, benign soft tissue mass that is commonly incorrectly diagnosed as a malign lesion due to its rapid and infiltrative growth pattern and histological characteristics. This case report discusses a 52-year-old female patient, who presented with a progressively growing mass of the right mandible within two months, resulting in numbness and tingling around the lips. Possibility of a malignant process was considered based on her complaints and clinical, radiologic, and pathologic findings. A complete excision of the mass that was adherent to the intraoperative marginal mandibular nerve indicated nodular fasciitis. As demonstrated by this case report, nodular fasciitis is a significant entity to consider when faced with similar clinical presentations.

Keywords: Cancer, nodular fasciitis, facial nerve

ÖZ

Nodüler fasiit, hızlı ve infiltratif büyüme paterni göstermesi ve histolojik özellikleri nedeniyle sıklıkla malignite tanısı konulabilen nadir görülen benign bir yumuşak doku kitlesidir. Bu olgu sunumunda, sağ çene kemiğinde iki ay içinde giderek büyüyen kitle yakınması ile başvuran ve dudaklarında uyuşma ve karıncalanma ortaya çıkan 52 yaşında bir kadın hasta tartışılmaktadır. Hastalığın şikayetleri ve klinik, radyolojik ve patolojik bulgularına göre malign bir süreç olduğu düşünüldü. Operasyon esnasında marjinal mandibular sinire yapışık olduğu görülen kitlenin total eksizyonu sonucunda nodüler fasiit tanısı konuldu. Bu olgu sunumunda belirtildiği gibi benzer klinik tablolar ile karşılaşıldığında, nodüler fasiit tanısı dikkate alınmalıdır.

Anahtar Kelimeler: Kanser, nodüler fasiit, fasiyal sinir

Introduction

Nodular fasciitis (NF) is a benign condition that can both clinically and histologically mimic more insidious and sarcomatous lesions. While the pathogenesis of NF has been suggested to be traumatic, infectious, or inflammatory, the role of these factors in the development of lesions is uncertain (1,2). Diagnosis is reported to be mainly between the third and fourth decades, and predominantly in males. In approximately 20% of the cases, NF is localized in the head and neck region. It generally presents as a unilateral painless mass which typically develops within a few weeks in subcutaneous or deeper soft tissue. The diagnosis of NF may be difficult due to various clinical and radiologic findings and troubling histological characteristics (1,3). This article presents an NF case with a rapidly growing lesion of the mandible, resulting in numbness in the lip, and thus being mistaken for a malign lesion based on the clinical examination and also radiological and histopathological findings.

Case Report

A 52 year-old female patient presented with complaints of swelling on the right mandible, which had progressively increased over two months, and numbness and tingling around the lips on the right side. The patient had no history of medication, alcohol or nicotine use, trauma or inflammation in the region, andsystemic disease. In palpation examination a rigid, fixated mass of about 20x10 millimeter (mm) was certainedon the right-side of the mandibular corpus. Facial nerve examination was unremarkable. Subsequent ultrasound has shown a 17x11 mm sized solid mass (suspicious for malignancy) on the anterior of the mandibular bone.

Consequent contrast-enhanced maxillofacial computerized tomography (CT) revealed a 16x11 mm soft tissue mass that was 4 mm under the skin, with homogeneous contrast and prominent benign lymph nodes in the neck. Fine needle aspiration cytology showed highly cellular smears with a polymorphic appearance (Figure 1). The cells were large and fibroblast-like with well-defined cytoplasmic borders. The nuclei showed pleomorphism with fine, evenly distributed chromatin and prominent nucleoli in many of the cells. A few scattered lymphocytes and neutrophils were present. The background was slightly myxoid. However, excision was recommended for definitive diagnosis. As a preliminary diagnosis of malignancy, fluorodeoxyglucose (18F-FDG) positron emission tomography (PET)-CT was performed (Figure 2). The maximum standardized uptake value (SUV$_{max}$) of the mass was 12.8. There were benign lymph nodes in the cervical chain. The patient was discussed in multidisciplinary oncology board and they recommended a complete excision of the mass under general anesthesia. During surgery, we noted that the mass located superiorly and very close to the marginal mandibular nerve (Figure 3). The marginal mandibular nerve was preserved as the mass was dissected from the mandible's periosteum and completely removed. No postoperative complication was observed. Histopathology showed benign spindle cells arranged in sheets and fascicles containing moderate-to-abundant fuzzy cytoplasm. Nuclear atypia was absent. Extravasated red blood cells (RBCs) were seen at occasional places with lymphocytic infiltrate. Immunohistochemically, the cellular component displayed reactivity toward vimentin (a fibroblast marker), calponin-b, and smooth-muscle specific actin (Figure 4). The spindle

cells of this lesion lack desmin, keratin, CD34, p53, or S100, which aids in securing the diagnosis of this proliferative but benign lesion over the more troubling items on the differential. Based on these findings, it was diagnosed as a NF. The patient remained free of disease at 15 months after surgery. The patient signed an informed consent before the investigation.

Discussion

NF was first characterized and named as pseudosarcomatous fibromatosis by Konwaler et al. (4). Since then, it has had various names such as pseudosarcomatous fasciitis, pseudosarcomatous fibromatosis, proliferative fasciitis, and infiltrative fasciitis. NF was often classified as some form of sarcoma, usually liposarcoma, fibrosarcoma, or rhabdomyosarcoma, before being described as a distinct mass (2).

NF pathogenesis seems to be reactive or inflammatory, involving fibroblastic or myofibroblastic proliferation, rather than truly neoplastic. Some studies suggest that local trauma may induce myofibroblast proliferation. As outlined in case reports of pregnant and lactating women with NF, another assumption is that estrogen receptor stimulation in myofibroblasts may lead to proliferation of those cells (1,5). In our case, the patient had no history of trauma, inflammation, or pregnancy, which suggests that there are some other factors in the pathogenesis of NF that are still unknown.

NF is incorrectly diagnosed due to clinical findings and radiological characteristics similar to malignant lesions.
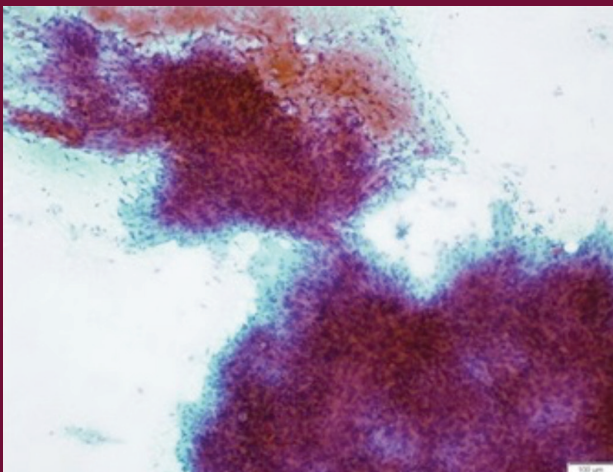

Figure 1. FNAC smear PAP stain 100^x (above) cellular smear showing predominantly spindle-shaped cells with low nuclear atypia
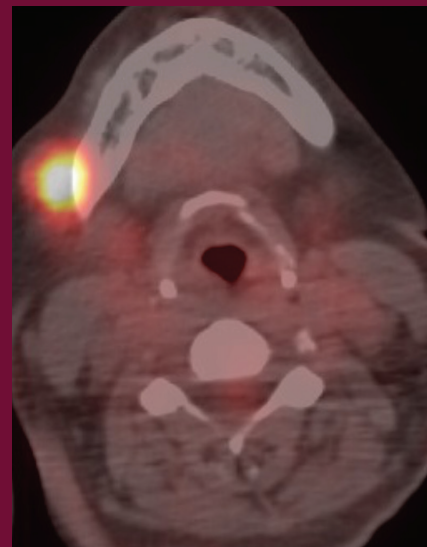
FNAC: Fine needle aspiration cytology, PAP: Papanicolaou

Figure 2. PET/CT with 18F-FDG demonstrates a focal area of uptake by the mandible with an SUV$_{max}$ of 12.8

PET/CT: Positron emission tomography/computed tomography, 18F-FDG: fluorodeoxyglucose

These masses are often misdiagnosed as squamous cell carcinomas, sarcomas, or uncommonly as metastases of other primary tumors. Morbidity of radical surgical procedures can be limited with preoperative diagnosis of this benign tumor (6).

For differential diagnosis, various imaging methods such as CT, magnetic resonance imaging, direct radiography, ultrasound, PET-CT can be performed preoperatively. NF has nonspecific and variable imaging characteristics. In the head and neck, depending on predominant stromal elements, NF may appear as a distinct solid or cystic mass. Lesions may be localized within the subcutaneous space, deeply situated along deep

Figure 3. Tumor on the mandible adhering to the marginal mandibular nerve

fascia, or embedded within muscle (7). 18F-FDG PET-CT is commonly used for benign/malign differentiation, as well as staging/restaging of various malignances by revealing glucose metabolism (8). However, increased 18F-FDG uptake may also manifest in some benign conditions, including abscess, pulmonary granuloma, tuberculosis, and sarcoidosis. Therefore, false-positive conditions are likely in a clinical setting (9). In our case, high SUV_{max} value led us to believe that the tumor was malignant.

NF must be clearly distinguished from spindle-cell sarcoma based on the degree of cytologic atypia, prominent in spindle-cell sarcoma. Fibromatosis (desmoid tumor), with dense collagenous stroma and absence of both myxoid areas and extravasated RBCs must also be ruled out (1). Dermatofibroma also shows spindle cell proliferation admixed with epithelioid histiocytes, but also lacks prominent vasculature and RBC extravasation. Proliferative fasciitis is characterized by ill-defined tumor growing along the fibrous septa with large myofibroblasts admixed with immature fibroblast-like spindle cells in a myxoid or collagenous background stroma, but absence of extravasated RBCs. Another differential diagnosis of NF includes benign nerve sheath tumors such as schwannoma, with Antoni A and Antoni B growth patterns, and neurofibroma, with abundant collagen and scant myxoid material, also lacking RBC extravasation (6).

Lenyoun et al. (10) indicated that NF only requires marginal excision without concern of recurrence, and Hseu et al. (3) reported that none of the patients who underwent complete resection developed recurrence. In our patient, who also underwent complete excision, the tumor was adherent to the marginal mandibular nerve and nerve was expanded. On the basis of these outcomes, we strongly recommend complete
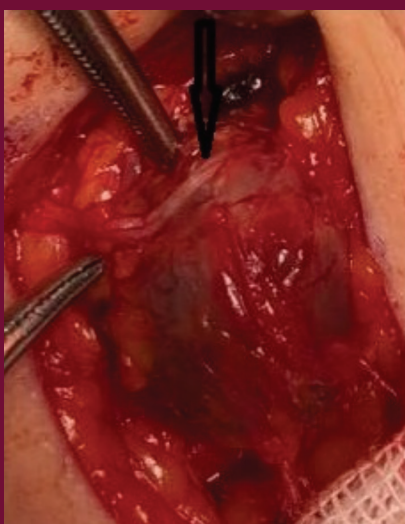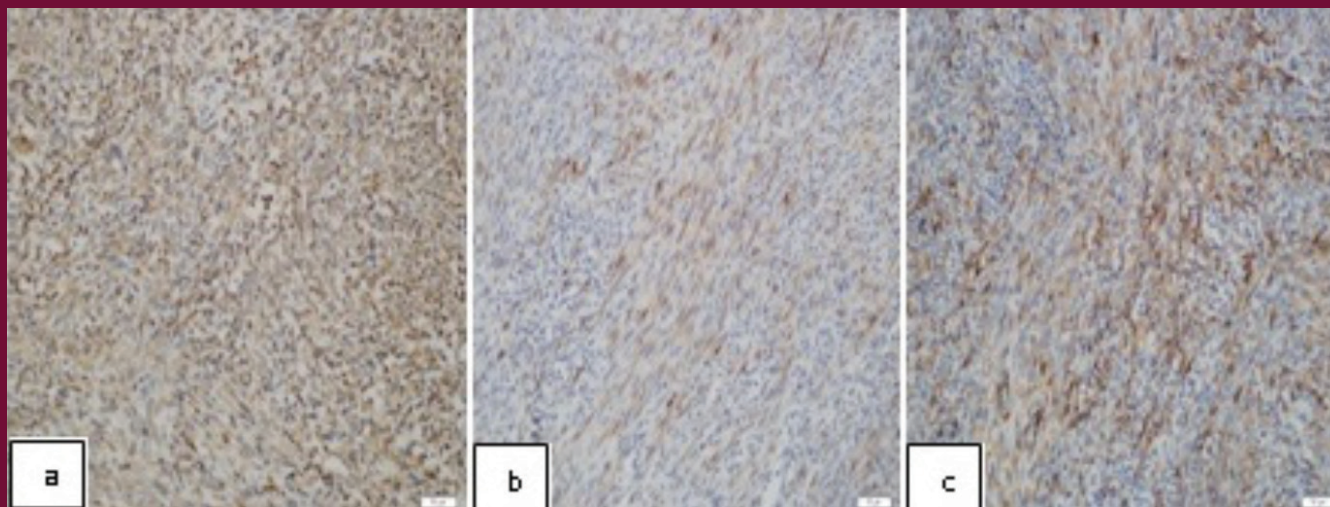

Figure 4. Wide spread positivity for vimentin (a), positive staining of fibroblasts, myofibroblasts for calponin-B (b), and smooth muscleactin (c) stains in sub-membranous "tram-track" pattern characteristic of myofibroblasts

local excision. Furthermore, localization of the mass must be considered because an imprecise procedure may cause an unwanted outcome such as facial paralysis. The patient had no sign of recurrence at 15 months after surgery.

Conclusion

In regard to the clinical and histopathological similarities of the NF, it can be easily misdiagnosed as a malignant tumor. NF should be in mind in the differential diagnosis of the masses in the head and neck area. Aggressive treatment should be avoided in patients who are considered to be diagnosed with NF.

Ethics

Informed Consent: The patient signed an informed consent before the investigation.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: S.B., N.K.T., B.E.E., İ.E.Ç., Concept: S.B., N.K.T., B.E.E., İ.E.Ç., Design: S.B., N.K.T., B.E.E., İ.E.Ç., Data Collection or Processing: S.B., N.K.T., B.E.E., İ.E.Ç., Analysis or Interpretation: S.B., N.K.T., B.E.E., İ.E.Ç., Literature Search: S.B., N.K.T., B.E.E., İ.E.Ç., Writing: S.B., N.K.T., B.E.E., İ.E.Ç.

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Hidden and Uncontrolled Hypertension Leading Aortic Dissection

Aort Diseksiyonuna Neden Olan Gizli ve Kontrolsüz Hipertansiyon

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ABSTRACT

Aortic dissection (AD) is one of the most devastating acute cardiovascular disorders and commonly occurs in patients with underlying aortic dilatation and connective tissue disorders such as Marfan syndrome and vascular type Ehlers-Danlos syndrome. In this report, we present a patient whose AD occurred in a normal aortic dimension due to underlying long-lasting, hidden and uncontrolled hypertension.

Keywords: Aortic dissection, left ventricular hypertrophy, hypertension

ÖZ

Aort diseksiyonu (AD) en tehlikeli akut kardiyovasküler hastalıklardan biri olup genellikle altta yatan aort dilatasyonu ve Marfan sendromu, vasküler tip Ehler-Danlos sendromu olan hastalarda gelişir. Bu yazıda normal aort çapına rağmen gizli, uzun süren ve kontrolsüz hipertansiyona bağlı olarak AD gelişen bir hastayı sunmaktayız.

Anahtar Kelimeler: Aort diseksiyonu, sol ventrikül hipertrofisi, hipertansiyon

Introduction

High blood pressure (BP), which is estimated to be seen in 30-45% of adults, is one of the most important reasons for cardiovascular mortality (1). It brings about an increased cumulative cardiovascular risk (hemorrhagic stroke, ischemic stroke, myocardial infarction, sudden death, heart failure, and peripheral artery disease) and end stage renal disease if untreated (2). Hypertension-mediated organ damage (HMOD), which means structural or functional changes in arteries or end organs (heart, blood vessels, brain, eyes, and kidney), caused by an elevated BP is a marker of pre-clinical or asymptomatic cardiovascular disease (3). Some types of HMOD can be reversed by antihypertensive treatment, especially when treated early, but with long-standing hypertension, HMOD may become irreversible despite improved BP control (4). Left ventricle hypertrophy, HMOD of the heart, could not be regressed or stopped despite diagnosed and treated timely. Approximately 1% of the hypertensive patients encounter with serious hypertensive urgency and/or hypertensive emergency (5). One of these hypertensive urgencies is aortic dissection (AD), which is one the most serious acute cardiovascular situations. The incidence of AD is 3/100,000 every year and most of these patients have a diagnosis of hypertension before the incident and this complication is commonly encountered in patients aged over 50 years (6,7). Some predictors of death in AD are hypotension, cardiogenic shock, pericardial tamponade, acute kidney failure, age >70-year, sudden onset of chest pain and abnormal changes in electrocardiography (8,9). Therefore, AD is rarely seen in young patients and newly diagnosed with hypertension. By presenting this case, we would like to demonstrate what a strengthened (thickened) left ventricle and long-lasting uncontrolled hypertension are capable to; an AD.

Case Report

A 54-year-old lady was brought to our emergency department (ED) by 112 ambulance due to syncope and chest pain that occurred while she was cleaning her bathroom. Her family

members suspected that she could have inhaled bleacher during the cleaning. Her past medical history was significant with newly diagnosed hypertension without additional cardiovascular risk factor. She was admitted to our ED with hypertensive urgency three months earlier, in which she was diagnosed with hypertension and treated as outpatient with oral antihypertensive drugs. She was prescribed metoprolol 50 mg once daily and quinapril + hydrochlorothiazide 20/25 mg once daily. At physical examination, she had a systolic BP of 80 mmHg and diastolic BP of 40 mmHg. Her heart rate was 109 bpm, temperature was 37.9 °C and respiratory rate was 19. Her electrocardiogram showed sinus tachycardia with ST segment depression in DI, AVL, V5 and V6. Her Glascow Coma score was 14 with loss of orientation to time, place, and person. She had a troponin I level of 431 pg/mL (0-11.6) without any abnormalities in complete blood count and biochemistry parameters. The bedside transthoracic echocardiogram showed flap images within the ascending aorta and pericardial effusion. The computerized tomography (CT) of the thorax revealed that ascending aorta dimension was in normal range (39 mm) and confirmed dissection flap in the ascending aorta (Figure 1A). The sagittal view of the CT showed dissection flap in aortic arc and extending hematoma to the thoracic aorta (Figure 1B). The transverse view of the CT showed a hypertrophic left ventricle with a posterior wall of 23 mm in diameter and an interventricular septum of 21 mm in diameter (Figure 1C). Besides, there was an effusion of 20 mm in diameter in the pericardium compressing to the right ventricle (Figure 1C). Unhappily, the patient died while being transferred for surgery.

Discussion

Chronic hypertension, in other words "the silent killer", is mainly asymptomatic. An acute symptomatic elevation in BP is referred to as hypertensive crisis which covers hypertensive emergency, hypertensive urgency and asymptomatic hypertension (10). Togetherness of high BP and symptoms of end-organ damage (headache, blurry vision, chest pain, shortness of breath, altered mental status, epistaxis, and oliguria) is called hypertensive emergency and requires immediate treatment (10). On the other hand, hypertensive urgency is defined as having a BP ≥180/110 mmHg without end-organ damage.

It was estimated that 8 million hospitalization and 20 million visits to ED are because of an acute hypertensive episode (11). Most of the cases are treated and released on the same day while some of them require more attention (12). Our case presented with hypertensive urgency three months earlier and was treated and released since there were no end-organ damage signs. Oral treatment was prescribed; cardiology and internal medicine examinations were advised. However, the patient could not follow the suggestions. Unfortunately, she presented with a life-threatening complication of hypertension, AD.

The morphology of AD is the delamination of the vessel wall starting from an "intimal tear" and formation of a "false lumen". However, the intimal tear could extend to the adventitia resulting in blood extravasation to a cavity (pericardial, pleural, or abdominal cavity). Although the main mechanism is yet to be described, a two-stage approach could be applied to make it lighten: medial degeneration

Figure 1. A) Thorax CT revealed that ascending aorta dimension was in normal range (39 mm) and confirmed dissection flap in the ascending aorta. The sagittal view of the CT showed dissection flap in aortic arc and extending hematoma (asterix) to the thoracic aorta. B) The sagittal view of the CT showed dissection flap in aortic arc and extending hematoma (asterix) to the thoracic aorta. C) The transverse view of the CT showed a hypertrophic left ventricle with a posterior wall of 23 mm in diameter and an interventricular septum of 21 mm in diameter. Besides, there was an effusion of 20 mm in diameter in the pericardium compressing to the right ventricle
CT: Computerized tomography

and mechanical wall stress. In Marfan syndrome and Vascular type Ehlers-Danlos syndrome, loss of elastic fibers and interconnecting elastic fibers cause medial aortic degeneration (13). The blood flow is presumed to be the trigger leading the "intimal tear" by producing shear stress on the aortic wall. Hypertension is present as a risk factor for AD in 50-86% of the cases (14,15,16). By decreasing blood flow of the vasa vasorum that nourishes one-third of the external aortic media, hypertension causes ischemia and damage and decreases elasticity (17). Besides, it acts as the "trigger" for entry site by increasing mechanical wall stress.

Our case was alone in terms of the AD in her family. None of her family members has aortic aneurysm and/or diagnosed with connective tissue disorders. Based on the CT findings, it could be assumed that she had an uncontrolled hypertension for a long time because her left ventricle wall thickens was 23 mm. AD might prove the serious side effect of uncontrolled hypertension despite a normal aortic dimension in this case.

Ethics

Informed Consent: The patient's relatives gave consent for this case report.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.K., M.K., G.C., C.Ö., O.K., Concept: A.K., M.K., G.C., C.Ö., O.K., Design: A.K., M.K., G.C., C.Ö., O.K., Data Collection or Processing: A.K., Literature Search: A.K., M.K., G.C., C.Ö., O.K., Writing: A.K., M.K., G.C., C.Ö., O.K.

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Unexpected Finding in an Adult Patient Without Typical Symptoms: Huge Pseudoaneurysm Fulfilled with Thrombus

Tipik Semptomları Olmayan Yetişkin Bir Hastada Beklenmedik Bulgu: Trombüs ile Dolu Dev Psödoanevrizma

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ABSTRACT

Although most patients with left ventricle (LV) pseudoaneurysm present with chest pain, dyspnea, signs and symptoms of congestive heart failure or peripheral embolism, a few cases with a huge LV pseudoaneurysm might present without typical symptoms. In this case, we reported a huge LV pseudoaneurysm fulfilled with thrombus in an adult patient who presented without specific symptoms. A 60-year-old male patient presented to outpatient clinic with symptom of fatigue. Transthoracic electrocardiograph examination suggested a pseudoaneurysm of posterior wall of the LV, but the quality of image was poor. Cardiac magnetic resonance imaging was performed providing the presence of huge pseudoaneurysm fulfilled with thrombus on the posterolateral side of the LV. With the cardiac operation, the LV pseudoaneurysm was resected and huge thrombus was retrieved in a single piece. This case illuminates that clinicians should be aware of atypical presentation in some patients with huge LV pseudoaneurysm.

Keywords: Huge, pseudoaneurysm, thrombus

ÖZ

Sol ventrikül (SV) psödoanevrizması olan hastaların çoğunda göğüs ağrısı, nefes darlığı, konjestif kalp yetmezliği veya periferik emboli belirtileri ve semptomları olsa da, büyük bir SV psödoanevrizması birkaç olguda tipik semptomlar olmadan da ortaya çıkabilir. Bu olguda, tipik semptomları olmayan yetişkin bir hastada trombüs ile dolu dev bir SV psödoanevrizmasını sunduk. Altmış yaşında erkek hasta yorgunluk şikayeti ile polikliniğe başvurdu. Transtorasik ekokardiyografik inceleme SV arka duvarında psödoanevrizma ile uyumlu idi, ancak görüntü kalitesi zayıftı. Yapılan kardiyak manyetik rezonans görüntülemede SV posterolateral tarafında trombüs ile dolu büyük psödoanevrizma izlendi. Kalp ameliyatı ile SV psödoanevrizması rezeke edildi ve tek bir parça halinde büyük trombüs alındı. Bu olgu, klinisyenlerin dev SV psödoanevrizması olan bazı hastalarda atipik sunumun farkında olmaları gerektiğini göstermektedir.

Anahtar Kelimeler: Dev, psödoanevrizma, trombüs

Introduction

Left ventricle (LV) pseudoaneurysm is described as a contained sac of the myocardium, which usually develops due to acute transmural myocardial infarction (1). Although most patients with LV pseudoaneurysm present with chest pain or dyspnea or signs and symptoms of congestive heart failure or peripheral embolism, a sizable portion of such cases might be diagnosed without typical symptoms (2). In this case, we reported a LV pseudoaneurysm in an adult patient without specific symptoms.

Case Report

A 60-year-old male patient presented to outpatient clinic with symptom of fatigue. The patient had a history of coronary artery stenting due to acute myocardial infarction two years

ago. Electrocardiography demonstrated a normal sinus rhythm, pathological Q wave in III and AVF leads without ST elevation, and T wave negativity in I and AVL leads (Figure 1A). Posteroanterior chest scan did not reveal any remarkable finding (Figure 1B). He underwent a computed tomography (CT) scan of the chest due to the suspicion of Coronavirus-2019 disease. On CT, it was found that there was a huge mass on the lateral side of the LV with a diameter of 5x4 cm (Figure 2A). To clarify the diagnosis, cardiac magnetic resonance imaging was performed providing the presence of huge pseudoaneurysm fulfilled with thrombus on the posterolateral side of the LV (Figure 2B). Transthoracic echocardiographic examination showed a LV ejection fraction of 40% and pseudoaneurysm of

posterior wall but the quality of image was poor. The cardiac operation, including the resection of huge pseudoaneurysm, was planned due to the risk of peripheral embolism. The coronary angiography before cardiac surgery revealed a total occlusion of the circumflex artery after obtuse marginal artery-2 (Figure 3A). The aneurysm was diagnosed as a pseudoaneurysm because there was a discontinuation, which suggests a rupture in the wall. The most lateral wall of the aneurysm could not be imaged because of technical difficulties (Figure 3B). During cardiac operation, it was noted that pseudoaneurysm was completely fulfilled with thrombus (Figure 4A). The pseudoaneurysm was resected and huge thrombus was retrieved in a single piece (Figure 4B).
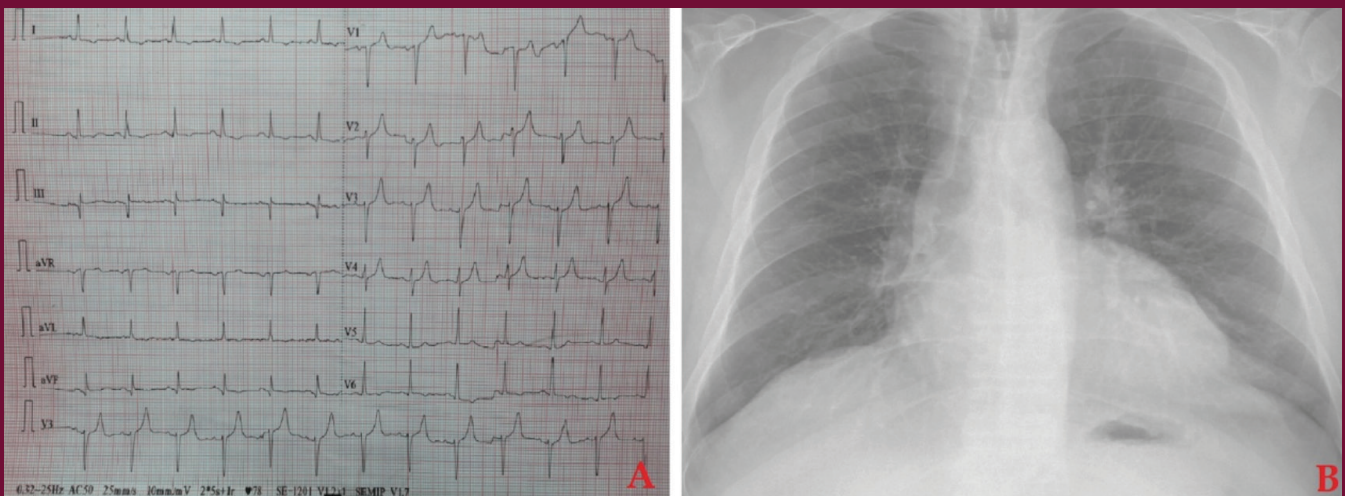

Figure 1. A) Electrocardiography of the case, B) Posteroanterior chest scan of the case
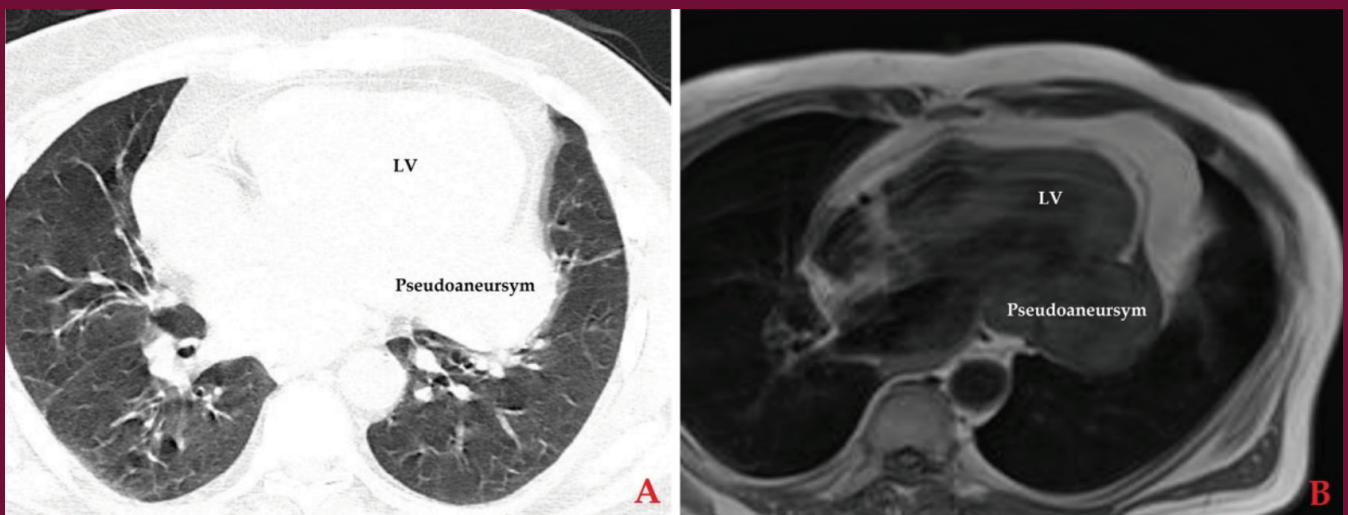

Figure 2. A) Computed tomography scan showing a mass on the LV, B) Cardiac MRI showing pseudoaneurysm on the posterolateral side of the LV
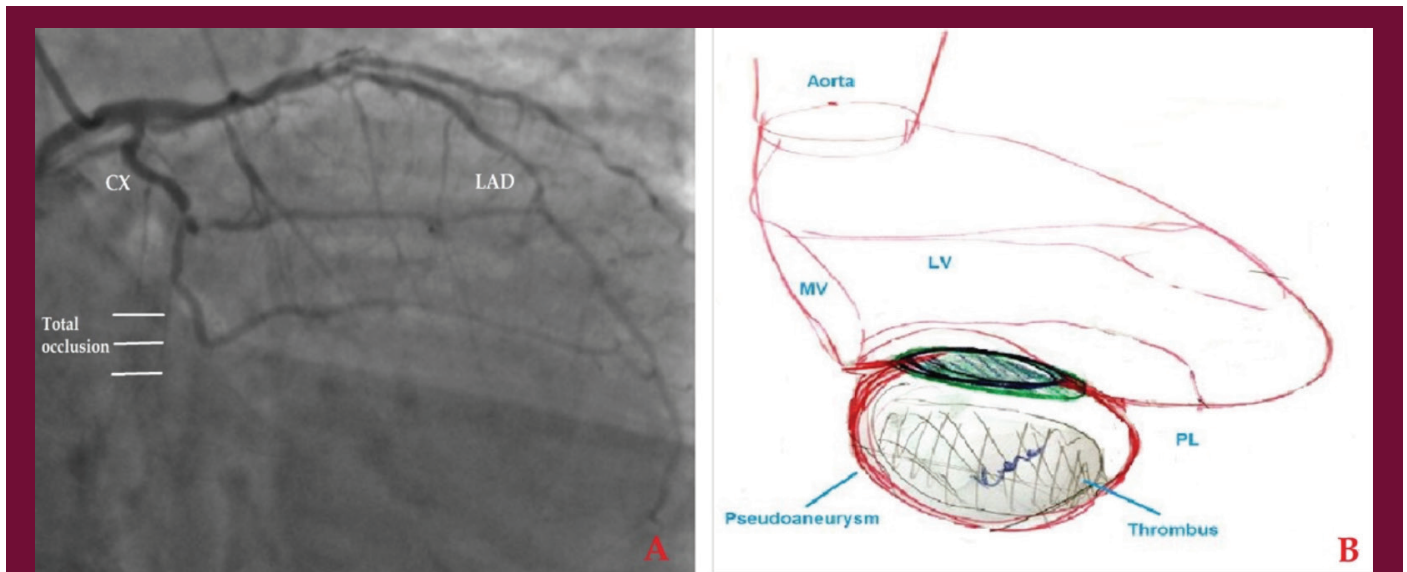*LV: Left ventricle, MRI: Magnetic resonance imaging*

Figure 3. A) CAG showing a total occlusion of CX artery after OM-2 artery, B) Schematic diagram of the LV pseudoaneurysm
CAG: Coronary angiography, CX: Circumflex artery, LV: Left ventricle, LAD: Left anterior descending artery, MV: Mitral valve, PL: Posterolateral

Figure 4. A) Thrombus fulfilled LV pseudoaneurysm, B) Gross specimen of LV pseudoaneurysm and huge thrombus
LV: Left ventricle

Discussion

LV pseudoaneurysm, which usually develops following cardiac rupture due to transmural myocardial infarction, has a small, akinetic wall consisting of mainly two layers of pericardium and fibrous tissue that is contrast to true aneurysms composed of three layers, including endocardium, myocardium, and pericardium (1,3). In our case, there was a discontinuity in the posterior wall and the wall of the aneurysm was very thin, suggesting pericardium instead of myocardial layers. The LV pseudoaneurysms have high risk of mortality because of a significant risk of sudden rupture. However, in this case, the thrombus might have prevented rupture of the wall of the pseudoaneurysm. In contrast to our case who presented without typical symptoms, most patients with LV pseudoaneurysm usually present with the signs and symptoms of heart failure, peripheral embolism, life threating arrhythmias, and chest pain (3). Therefore, the clinicians should be aware of atypical presentation in some patients with LV pseudoaneurysm. As shown in our case, in patients who presented with huge LV pseudoaneurysm

fulfilled with thrombus should undergo a cardiac surgery, including the resection of pseudoaneurysm and retraction of thrombus, due to the risk of peripheral embolism (4).

Ethics

Informed Consent: Informed consent was obtained from the patient for publishing this case report.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: S.D., T.Ç., Design: S.D., T.Ç., M.K., İ.A., M.U., Data Collection or Processing: S.D., T.Ç., M.K., İ.A., M.U., Analysis or Interpretation: S.D., T.Ç., M.U., Literature Search: M.K., İ.A., Writing: S.D., T.Ç., M.U.

Conflict of Interest: No conflict of interest was declared by the authors.

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