JAK-2 V617F Mutation in Endothelial Cells of Patients with Atherosclerotic Carotid Disease

Diz-Küçükkaya R. et al: *JAK-2 V617F* Mutation in Endothelial Cells

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Abstract

Objective: It has been shown that clonal mutations occur in hematopoietic stem cells with advancing age and increase the risk of death due to atherosclerotic vascular diseases, just like in myeloproliferative neoplasms. It is known that endothelial cells (EC) and hematopoietic stem cells develop from a common stem cell called hemangioblast in early embryonic period. However, the presence of hemangioblast in the postnatal period is controversial. In this study, *JAK2* gene variants was examined in patients with atherosclerotic carotid disease and without any hematological malignancy.

Materials and Methods: Ten consecutive patients (8 men and 2 women) with symptomatic atherosclerotic carotid stenosis were included in this study. EC (CD31$^+$CD45$^-$) were separated from tissue samples taken by carotid endarterectomy. *JAK2* variants was examined in EC, peripheral blood mononuclear cells and oral epithelial cells of the patients with next generation sequencing.

Results: The median age of the patients was 74 (58-80) and the median BMI was 24,44 (18,42-30,85) kg/m². Smoking history was present in 50%, hypertension in 80%, diabetes in 70%, and ischemic heart disease in 70% of the patients. *JAK2V617F* mutation was detected in peripheral blood mononuclear cells in three out of 10 patients, two of them also had *JAK2V617F* mutation in their EC. *JAK2V617F* mutation was not found in oral epithelial cells in any of the patients.

Conclusion: In this study, for the first time in the literature, we showed that *JAK2V617F* mutation was found somatically in both peripheral blood cells and EC in patients with

atherosclerosis. This finding may support that EC and hematopoietic cells originate from a common clone or that the somatic mutation can be transmitted to EC by other mechanisms. Examining the molecular and functional changes caused by *JAK2V617F* mutation in EC may help open a new avenue for treating atherosclerosis.

Keywords: JAK2V617F mutation, clonal hematopoiesis, atherosclerosis, myeloproliferative neoplasms.

Öz

Amaç: Yaşla birlikte hematopoietik kök hücrelerde klonal mutasyonların ortaya çıktığı, aynı miyeloproliferatif neoplazilerde olduğu gibi, bu mutasyonların aterosklerotik damar hastalıkları ile ölüm riskini arttırdığı gösterilmiştir. Embriyonik hayatta erken dönemde endotel ve hematopoietik kök hücrenin hemangioblast adı verilen ortak bir kök hücreden geliştiği bilinmektedir. Ancak postnatal dönemde hemangioblast varlığı tartışmalıdır. Bu çalışmada herhangi bir hematolojik malignitesi olmayan karotis aterosklerozlu hastalarda *JAK2* gen varyantları incelenmiştir.

Gereç ve Yöntemler: Bu çalışmaya semptomatik aterosklerotik karotis darlığı olan ardışık 10 hasta (8 erkek, 2 kadın) dahil edilmiştir. Karotis endarterktomi operasyonu ile alınan doku örneklerinden endotel hücreleri (CD31$^+$CD45$^-$) ayrılmıştır. Hastaların endotel hücreleri, periferik kan mononükleer hücreleri ve ağız epitel hücrelerinde *JAK2* varyantları yeni nesil dizileme ile incelenmiştir.

Bulgular: Hastaların ortanca yaşı 74 (58-80), ortanca BMI 24,44 (18,42-30,85) kg/m^2 idi. Sigara öyküsü %50, hipertansiyon %80, diyabet %70, iskemik kalp hastalığı %70 hastada mevcuttu. Genetik analizde 10 hastanın üçünde periferik kan mononükleer hücrelerinde *JAK2 V617* mutasyonu saptandı. Bu 3 hastanın ikisinde endotel hücrelerinde de *JAK2 V617* mutasyonu gösterildi. Hastaların hiç birinde ağız epitel hücrelerinde *JAK2 V617* mutasyonuna rastlanmadı.

Sonuç: Bu çalışmada, literatürde ilk defa, herhangi bir hematolojik malignitesi olmayan ateroskleroz hastalarında hem periferik kan hücrelerinde, hem de aterosklerotik plaktan izole edilen endotel hücrelerinde somatik olarak *JAK2 V617* mutasyonu bulunduğu gösterilmiştir. Bu bulgu endotel hücreleri ve hematopoietik hücrelerin ortak bir klondan kaynaklandığını veya somatik mutasyonun başka mekanizmalarla endotel hücrelerine iletilebildiğini destekleyebilir. Endotel hücrelerinde *JAK2 V617* mutasyonunun yarattığı moleküler ve fonksiyonel değişikliklerin incelenmesi, ateroskleroz tedavisinde yeni bir sayfanın açılmasına yardımcı olabilir.

Anahtar Sözcükler: JAK2V617F mutasyonu, klonal hematopoiezis, aterosklerozis, miyeloproliferatif neoplaziler.

INTRODUCTION

Atherosclerosis is a chronic vascular disease characterized by the accumulation of oxidized low-density lipoproteins (ox-LDL) in the intimal layer of the arteries leading to inflammatory reactions [1]. Atherosclerotic vascular diseases (myocardial infarction, stroke, and peripheral artery occlusion) are the leading cause of death for humans worldwide, causing an estimated 17.9 million deaths each year according to the World Health Organization [2]. Apart from conventional risk factors for atherosclerosis such as obesity, smoking, hyperlipidemia, diabetes, and hypertension; atherosclerosis is further exacerbated by chronic inflammatory disorders and myeloproliferative neoplasms (MPN) [1,3].

MPN are a heterogenous group of hematological malignancies with a chronic course, characterized by abnormal proliferation of myeloid precursors as a result of somatic mutations

in the hematopoietic stem cells. MPN usually develops as a result of 'driver mutations' including *JAK2V617F*, *CALR*, and *MPL* [4]. The most important factor determining morbidity and mortality in MPN patients is thrombotic complications [5]. Increased red blood cell mass, activation of leukocytes and platelets, stimulation of coagulation and inflammation have been postulated as possible causes of thrombosis in patients with MPN. After the definition of mutation in the 'Janus Kinase-2' gene (*JAK2V617F* mutation), it has become clear that the mutation profile and allele burden also played an important role in the development of thrombosis [4,5]. *JAK2V617F* mutation generates more active neutrophils and monocytes, increases leukocyte-endothelial cell (EC) interactions, and causes more tissue factor expression and in turn induces thrombosis [6-8]. Most of the studies investigating the relationship between *JAK2V617F* mutation and thrombosis have focused on the functional changes of hematopoietic cells originating from the MPN clone, there are only a few studies on the EC in MPN patients. Sözer et al in 2009 [9] and Rosti et al in 2013 [10], isolated hepatic and splenic vein endothelial cells from MPN patients with splanchnic thromboses demonstrating the presence of *JAK2V617F* mutation in EC. It has been suggested that EC and hematopoietic stem cells originate from a common stem cell called 'hemangioblast' in the early embryonic stage. Sözer and Rosti interpreted the detection of a somatic *JAK-2 V617F* mutation in both EC and hematopoietic cells as evidence supporting the existence of the hemangioblast during the postnatal period in humans [9,10]. On the other hand, conflicting results have been obtained in other studies conducted with circulating endothelial progenitor cells, suggesting EC and hematopoietic cells originate from different stem cells and EC do not harbor *JAK2V617F* mutation in patients with MPN [11,12].

Large-scale studies with peripheral blood cells from normal populations showed that the frequency of somatic mutations increased with advancing age being around 10% after 70 years of age. Interestingly, most of these variants appeared to accumulate in genes frequently implicated in hematological malignancies such as *JAK2, DNMT3A* (DNA methyltransferase 3-alpha)*, and TET2* (Tet-methylcytosine dioxygenase-2) [13]. Individuals harboring a clonal hematopoietic mutation without any signs of hematological malignancy are referred to as having 'clonal hematopoiesis of indeterminate potential (CHIP)'. The risk of developing hematological malignancy during follow-up increases in individuals carrying CHIP mutations (0.5-1% /per year) as expected, but the increased risk of death (40%) largely comes from cardiovascular complications. Studies demonstrated that the presence of CHIP mutations increases the risk of coronary artery thrombosis by 1.8-4 times, premature coronary artery disease by 4, and ischemic stroke by 2.6 [14,16]. Animal studies also illustrated that these mutations cause aberrant inflammation and accelerate the atherosclerotic plaque burden [15,17].

While numerous studies have explored the role of CHIP mutations in hematopoietic cells in atherosclerosis, there is no data on the presence of these mutations in EC. This study investigates *JAK2* gene variants in EC of patients with atherosclerosis.

MATERIAL and METHODS:
Inclusion of the patients and sample collection
Patients older than 50 years of age who were presented with neurological symptoms, found to have carotid artery stenosis exceeding 70% in the angiography were consecutively included. All patients were evaluated by both a cardiologist and a hematologist. Patients with a history of malignancies including MPN, previous chemotherapy and/or immune-suppressive treatment and patients with thrombocytosis, erythrocytosis, and splenomegaly were excluded. Carotid endarterectomy operations were performed in Mehmet Akif Ersoy Chest and Cardiovascular Surgery Education and Research Hospital. Oral swab and blood samples of the patients were collected during pre-operative testing. Demographic and clinical data of the patients were obtained from their file.

Carotid endarterectomy operation

After the surgical incision, the 'common carotid artery-CCA' and its branches were found, an arteriotomy was performed starting from proximal part of the CCA bifurcation and extending towards the internal carotid artery. The atheroma plaque was gently cut and removed. Carotid artery closed with primary suture or patch angioplasty. Carotid endarterectomy materials were preserved in sterile Petri dishes added with tissue storage solution (MACS-Tissue Storage Solution, Miltenyi Biotech) (Figure 1).

Isolation of EC

Atheroma tissue was cut into small pieces of 0.5 mm with the help of a scalpel. The mixture was dissociated with Human Tumor Dissociation Kit and GentleMacs Octo Dissociator for 1 hour according to manufacturer's protocols (Miltenyi Biotec, Germany). The dissociated cell solution was passed through 40-micron and 70-micron strainers (Sartorius, USA), washed with PBS, centrifugated at 500xg for 10 minutes and an additional wash with 10 mL of PBS was performed afterwards. To remove erythrocytes, cells were incubated with 4 mL NH_4Cl erythrocyte lysate solution (Thermo Scientific, USA) for 10 minutes and centrifuged at 500xg for 5 minutes. The supernatant was discarded, and cells were incubated with 100ul PBS containing Zombie NIR viability dye (Biolegend, USA) for 10 minutes in dark. After incubation, the cells were washed and incubated with 100 μl of staining buffer (SB; PBS + %1 BSA) containing mouse anti-human CD31 PE-Dazzle 594 (Clone WM59, Biolegend USA) and mouse anti-human CD45 PE-Cy7 (Clone HI39, Biolegend USA) antibodies for 30 minutes in dark and washed at 500xg for 5 minutes. The supernatant was discarded, and pellet were resuspended with SB for sorting. Cells were sorted as live $CD45^-CD31^+$ cells via FACS Aria III (BD Biosciences, USA) according to the gating strategy given in Figure 2. Cells were sorted with 'purity' logic at a speed of ≤1500 events/second for maximum purity and efficiency. Live $CD45^-CD31^+$ cell purities were ≥95% after sorting.

NGS analysis for JAK2 variants

DNAs from EC, peripheral blood mononuclear cells and oral mucosal cells were isolated. Twist Exome 2.0 kit and Twist Human Core Exome Enzymatic Fragmentation (EF) Multiplex Complete kit (CA USA) was used for library construction, and MGIEasy FS DNA Library Prep Kit was performed for formation circular DNA and the library to be ready for sequencing on the MGI system. The library was sequenced on the (MGI-DNBSEQ-G400, China) instrument generating 150 bp paired-end read with 100X mean target coverage. The output of NGS was raw fastq files. Reads were aligned to the reference human genome (hg19). Variants were identified with Genemaster (Istanbul Turkey). Integrative Genomic Viewer (IGV) software was used for variants visualization.

Bioinformatics analysis

Next Generation Sequencing (NGS) paired-end data from 60 FASTQ files (R1.fq.gz / R2.fq.gz) were checked for quality scores across all bases by FastQC (Galaxy Version 0.74) analysis tool. Per base sequence quality was calculated for each sample including EC, peripheral blood and oral epithelial cells, respectively. Phred scores were obtained greater than 30 and sequencing libraries were considered to be a good quality for subsequent NGS downstream analysis. All publicly open-source algorithms were used through the European Galaxy server. Mapping of paired-end reads against reference human genome (Homo sapiens: hg38 Full) was conducted by Bowtie2 alignment tool (Galaxy Version 2.5.3). The outputs were generated as binary bam alignments file in BAM and BAI format. Integrative Genomics Viewer (IGV) Version 2.13.1 was used to visualize the sequence alignment data from each indexed BAM files. Pathogenic/likely pathogenic variants released by the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/), also single nucleotide variants (SNVs), insertions,

deletions and missense mutations were analyzed in each patient as trio manner (EC, blood cells, oral epithelial cells).

Statistical Analysis

Statistical analysis was performed using the Mann–Whitney U test for continuous variables and Fisher's exact test for categorical variables when evaluating the clinical and demographic data of the patients. Since statistical significance could not be achieved, eta squared or Cohen's d statistics were not performed to reveal statistical power. All statistical analyses were performed using IBM SPSS 27 software.

RESULTS:

Demographical features of the patients

In this study, 10 consecutive patients (8 male and 2 female) were included. The median age was 74 (58-80) (Table 1). None of these patients had splenomegaly, any sign of malignancy including hematological malignancies and MPN (Table 2).

Risk factors for atherosclerosis

The median BMI was 24.44 (18,42-30.85) kg/m^2. Smoking history was present in 5 patients, median daily cigarette consumption was 15 (5-30). Eight patients were diagnosed as hypertension, 7 had diabetes. Ischemic heart disease was diagnosed in 7 of the patients, and 1 had peripheral artery disease (Table 1).

***JAK2* mutation analysis**

When the *JAK2* gene was examined using NGS, we found the *JAK2V617F* mutation in the peripheral blood mononuclear cells of 3 out of 10 patients. We demonstrated the *JAK2V617* mutation in the EC of 2 of these 3 patients. Variant allele frequencies ranged between 0.43% and 3%. We did not find *JAK2V617F* mutation in oral epithelial cells in any patients (Table 3). No other pathogenic variants besides *JAK2V617* mutation were detected in our cohort. Patients positive for the *JAK2V617F* mutation were statistically compared with those without the mutation in terms of clinical and laboratory data. However, due to small sample size, no significant results were obtained.

DISCUSSION

In this study, the EC isolated from carotid endarterectomy materials of patients who had symptomatic carotid atherosclerosis but had no hematological malignancies were evaluated in terms of *JAK2* variations. We found *JAK2V617F* mutation in peripheral blood cells of 3 patients, and 2 of them had *JAK2V617F* mutation also in EC. Due to the small number of patients, it is not appropriate to provide a rate for the *JAK2V617F* mutation in our cohort. However, recent studies have shown a high frequency of *JAK2V617F* mutation in the general population and in various groups of patients with thrombosis. In the study by Kristiansen and colleagues, the *JAK2V617F* mutation was investigated in 538 stroke patients and 19,958 controls using highly sensitive droplet digital PCR. The prevalence of *JAK2V617F* mutation was found to be 11% in stroke patients and 4.4% in the control group [16].'

In the literature, *JAK2V617F* mutation has been shown in the EC of the hepatic and splenic veins in MPN patients with splanchnic thromboses [9,10]. However, some researchers have suggested that these cells may be endothelial-like cells developing from MPN clones, and that the EC and hematopoietic cells originated from different stem cells [11,12]. In our study, we isolated CD31+CD45- cells obtained from carotid atheroma plaques, which represents mature EC, not monocyte-derived endothelial-like cells. While the *JAK2V617F* mutation was also detected in the peripheral blood mononuclear cells, they were not present in oral epithelial cells. This finding suggests that the *JAK2V617F* mutation is not germ-line, and that the somatic *JAK2V617F* mutation develops together in hematological cells and EC. Studies in the literature examining the relationship between *JAK2V617F* or *TET2* mutations and atherosclerosis focused on monocytes and neutrophils originating from the mutated hematopoietic clones [17,18]. To the best of our knowledge, our study represents the first

demonstration of the *JAK2V617F* mutation in EC of patients with atherosclerosis but without hematological malignancies.

The occurrence of *JAK2V617F* mutation in both EC and hematopoietic cells can be explained by various hypotheses. It is controversial whether 'hemangioblasts' that constitute endothelial and hematopoietic stem cells still exist in the postnatal period since animal experiments failed to demonstrate the postnatal presence of hemangioblasts [19,20,21]. Our results may support that hemangioblasts exist in the postnatal period in humans and that the *JAK2V617F* mutation may develop at the hemangioblast level. On the other hand, it is not known exactly when and in which stem cell the *JAK2V617F* mutation occurred. The existence of data suggesting that the *JAK2V617F* mutation may be acquired in childhood, or even '*in utero*' in some rare cases may indicate that, at least in some patients, the mutation could develop during the 'prenatal hemangioblast stage' [22]. In the third hypothesis, it can be speculated that EC and hematopoietic cells originate from different stem cells, but clonal hematopoietic mutations are transferred to EC from clone-derived hematological cells. In both solid and hematological cancers, genetic abnormalities of the cancer cells (aneuploidi, cancer-specific mutations) have been also detected in the EC within the tumor tissue [23]. It has been suggested that EC, which do not originate from the same origin as cancer cells, acquire these genetic changes through gene transfer via cancer-derived microvesicles [23,24]. In an *in vitro* study performed by Hekimoğlu and colleagues, DNA fragments carrying the *JAK2V617F* mutation were detected in microparticles secreted from *JAK2V617F*-positive EC, indicating the potential transmission of the *JAK2V617F* mutation to neighboring and distant cells [25].

The current treatment strategy for atherosclerosis focuses primarily on lowering LDL levels, preventing platelet activation, and modifying personal risk factors. Despite this approach, atherosclerotic cardiovascular diseases continue to be the number one cause of death in all countries of the world. With the understanding of the role of inflammation in the pathogenesis of atherosclerosis, the use of anti-inflammatory drugs has garnered attention in recent years [3]. In a randomized controlled trial, canakinumab (a human monoclonal antibody that neutralizes of interleukin-1beta signaling and suppresses inflammation) was administered to patients with a history of myocardial infarction and with increased CRP (> 2 mg/L). Targeting interleukin-1beta with canakinumab resulted in a significantly lower rate of cardiovascular events, regardless of lipid-lowering [26]. When these patients were evaluated for somatic mutations, CHIP variants were detected in the peripheral blood in 8.6% of them. Subgroup analysis revealed that patients with *TET2* variations responded better to canakinumab in terms of reducing the frequency of cardiovascular events [27]. EC are major participants in and regulators of inflammatory reactions. Our study has revealed that in addition to hematopoietic cells, EC can participate in atherosclerosis by harboring *JAK2V617F* mutation. Considering the evidence indicating that the activation of the JAK-STAT signaling pathway by the *JAK2V617F* mutation in leukocytes enhances inflammatory responses [6-8], it is reasonable to anticipate that the presence of *JAK2V617F* mutation in EC will result in comparable inflammatory outcomes, potentially exacerbating the atherosclerotic process. Investigating in detail the functional changes caused by *JAK2V617F* mutation in EC will help in elucidating the pathogenesis of atherosclerosis and will help identify new treatment targets.

The major limitation of our study is the small number of patients. However, for the first time in this study EC from the carotid artery were analyzed for somatic mutations. By increasing the number of the patients, we plan to screen other CHIP variants in patients with atherosclerosis. Another limitation of the study is the lack of bone marrow biopsy evaluation in our patients, MPN was excluded based on clinical (absence of splenomegaly) and laboratory (blood counts and serum LDH levels) findings.

Conclusion

In our study, we observed that patients with atherosclerosis have *JAK2V617F* mutation not only in peripheral blood cells but also in EC taken from the atherosclerotic plaque. The absence of *JAK2V617F* mutation in the oral epithelial cells in these patients supports that *JAK2V617F* mutation developed somatically in the blood cells and EC. Examining the effects of somatic mutations on the EC may help open a new avenue for treating atherosclerosis.

Authorship:
Contribution: R.DK. designed and performed research, analyzed data, and wrote the paper; T.İ. enrolled patients, performed surgeries, and wrote the paper; Ö.A performed research in the laboratory and wrote the paper; C.E. performed bioinformatic analysis, and wrote the paper, T.G designed research, contributed analytical tools, and wrote the paper.

Conflict-of-interest disclosure:
The authors declare no competing financial interests related to this study.

References:
1. Libby P, Buring JE, Badimon L, et al. Atherosclerosis. Nat Rev Dis Primers 2019;5:56.
2. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1
3. Libby P. Inflammation and the pathogenesis of atherosclerosis. Vascul Pharmacol 2024;154:107255.
4. Tefferi A, Vannucchi AM: Genetic risk assessment in myeloproliferative neoplasms. Mayo Clin Proc 2017;92:1283-1290.
5. Falanga A, Marchetti M. Thrombosis in myeloproliferative neoplasms. Semin Thromb Haemost 2014;40:348-358.
6. Passamonti F, Rumi E, Pietra D, et al. Relation between *JAK2* (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34$^+$ cells into peripheral blood in myeloproliferative disorders. Blood 2006;107:3676–3682.
7. Arellano-Rodrigo E, Alvarez_Larran A, Reverter JC, et al. Platelet turnover, coagulation factors, and soluble markers of platelet and endothelial activation in essential thrombocythemia: Relationship with thrombosis occurrence and JAK2 V617F allele burden. Am J Hematol 2008;84:102-108.
8. Reeves BN, Beckman JD. Novel pathophysiological mechanisms of thrombosis in myeloproliferative neoplasms. Curr Hematol Malig Rep 2021;16: 304–313.
9. Sözer S, Fiel MI, Schiano T, Xu N, Mascarenhas J, Hoffman R. The presence of JAK2V617F mutation in the liver endothelial cells of patients with Budd-Chiari syndrome. Blood 2009;113:5246-5249

10. Rosti V, Villani L, Riboni R, et al. Spleen endothelial cells from patients with myelofibrosis harbor the JAK2V617F mutation. Blood 2013;121:360-368.
11. Yoder MC, Mead LE, Prater D, et al. Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. Blood 2007;109:1801-1809.
12. Piaggio, V Rosti, M Corselli, et al. Endothelial colony forming cells (ECFCs) from patients with chronic myeloproliferative disorders lack the disease-specific molecular clonality marker. Blood 2009;114:3127-3130.
13. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014;371:2488-2498.
14. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. N Engl J Med 2017;377:111-121.
15. Marnell CS, Bick A, Natarajan P. Clonal hematopoiesis of indeterminate potential (CHIP): Linking somatic mutations, hematopoiesis, chronic inflammation and cardiovascular disease. J Mol Cell Cardiol 2021;161:98-105.
16. Kristiansen MH, Kjaer L, Skov V, et al. JAK2V617F mutation is highly prevalent in patients with ischemic stroke: a case control study. Blood Adv 2023;7:5825-5834.
17. Fuster JJ, MacLauchan S, Zuriaga MA, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. Science 2017;355:842-847.
18. Fidler TP, Xue C, Yalcinkaya M, et al. The AIM2 inflammasome exacerbates atherosclerosis in clonal haematopoiesis. Nature. 2021;592:296-301.
19. Lacaud G, Kouskoff V. Hemangioblast, hemogenic endothelium, and primitive versus definitive hematopoiesis. Exp Hematol 2017;49:19-24.
20. Tsuji-Tamura K, Ogawa M. Morphology regulation in vascular endothelial cells. Inflammation and Regeneration 2018;38:25.
21. Farina M, Russo D, Hoffman R. The possible role of mutated endothelial cells in myeloproliferative neoplasms. Haematologica 2021;106(11):2813-2823.
22. Williams N, Lee J, Mitchel E, et al. Life stories of myeloproliferative neoplasms inferred from phylogenies. Nature 2022;602:162-168.
23. Hida K, Hida Y, Shindoh M. Understanding tumor endothelial cell abnormalities to develop ideal anti-angiogenic therapies. Cancer Sci 2008;99:459-466.
24. Kawamoto T, Ohga N, Akiyama K, et al. Tumor-derived microvesicles induce pro-angiogenic phenotype in endothelial cells via endocytosis. PLoS One 2012;7:e34045.
25. Hekimoğlu H, Toprak SF, Sözer S. JAK2 V617F-positive endothelial cells induce apoptosis and release JAK2 V617F-positive microparticles. Turk J Hematol 2022;39: 13-21.
26. Ridker PM, Everett BM, Thuren T, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Eng J Med 2017;377:1191-1131.
27. Svensson EC, Madar A, Campbell CD, et al. TET2-driven clonal hematopoiesis and response to canakinumab: an exploratory analysis of the CANTOS randomized clinical trial. JAMA Cardiol 2022;7:521-528.
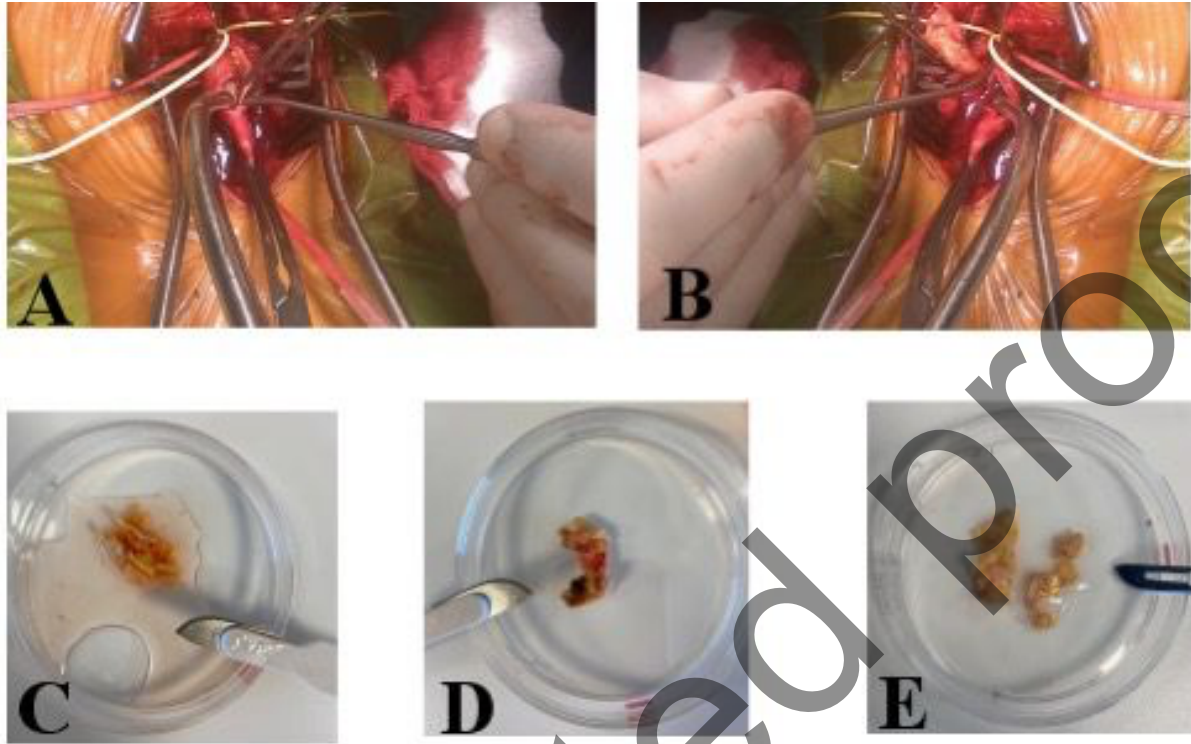
Figure 1: Carotid endarterectomy operation and ateroschlerotic plaque samples:
Exploration of carotid artery (A), and removal of the atherosclerotic plaque (B).
Atherosclerotic plaque materials rich in lipids and calcium (C-D-E), some of which contain
necrotic areas (D)
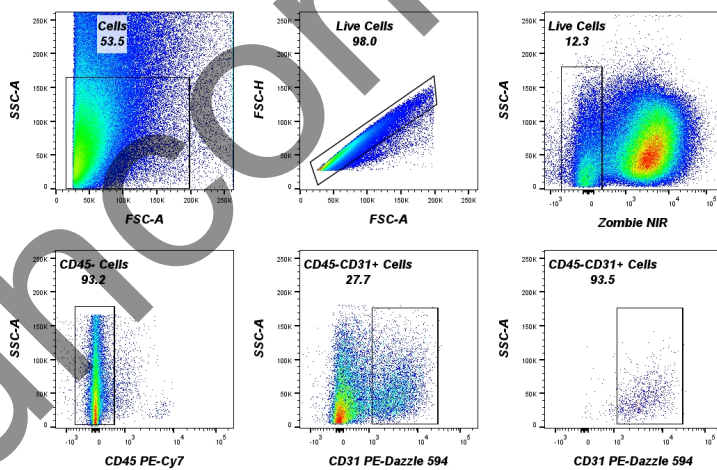
Figure 2-Isolation of Endothelial Cells by Flow Cytometry
This figure shows the gating strategy for sorting Endothelial Cells. **A)** Total cells from tissue
dissociate were gated in FSC-A x SSC-A plot and **B)** Doublets were discriminated using FSC-

A x FSC-H plot. **C)** Live cells were gated as Zombie NIR$^-$ cells. **D)** CD45$^-$ cells were selected to gate out leukocytes and **E)** CD31$^+$ cells were sorted. **F)** After sorting, CD45$^-$CD31$^+$ cells were analyzed to check the purity and efficiency of sorting process.

Table 1: Demographical features of the patients

NO	Sex	Age	BMI	Smoking	DM	HT	IHD	Cor. St.	Cor. ByPass
1-RK	M	74	18,42	+	-	+	-	-	-
2-HB	F	77	30,85	-	+	+	+	-	+
3-AT	**M**	**76**	**27,77**	-	+	+	+	+	+
4-MDA	M	67	25,82	+	+	+	+	-	+
5-RÖ	M	58	23,88	-	+	-	+	+	-
6-FS	F	73	26,96	+	+	+	+	-	+
7-OO	M	76	23,51	+	+	+	+	-	-
8-AK	**M**	**74**	**24,44**	+	-	+	+	+	-
9-İK	**M**	**80**	**23,66**	-	-	-	+	+	-
10-HE	M	73	30,64	-	+	+	-	-	-

Abbreviations: BMI: Body mass index, DM: Diabetes mellitus, HT: hypertension, IHD: Ischemic heart disease, Cor. St: Coronary stenting history, Cor. By-Pass: Coronary By-Pass operation history

Table 2: Blood count values and LDH levels of the patients

NO	WBC (x10⁹/L)	HB (gr/dL)	HCT %	PLT (x10⁹/L)	LDH (U/L)
1-RK	6,31	13,3	40,1	194	165
2-HB	6.68	13,1	41,4	319	210
3-AT	**5,97**	**14,2**	**43,6**	**156**	**204**
4-MDA	9.84	14,3	41,7	250	177
5-RÖ	6,4	14,8	42,8	204	163
6-FS	5,03	14,1	41	247	198
7-OO	9,26	15,5	47,9	155	218
8-AK	**8,8**	**13,9**	**44,2**	**194**	**220**
9-İK	**7,82**	**13,8**	**42**	**161**	**187**
10-HE	4,99	11,9*	36*	168	156

Abbreviations: WBC: white blood cells, HB: hemoglobin, HCT: hematocrit, PLT: platelets, LDH: lactate dehydrogenase (normal reference values between 90-240 U/L). Bold lines belong to JAK-2 V617F mutation positive patients.
*This patient had iron deficiency anemia due gastric ulcer.

Table 3: *JAK2 V617F* variant analysis of the patients

NO	Endothelial Cells – JAK2-V617F Genotype	Peripheral Blood Cells-JAK2-V617F Genotype (VAF %)	Oral Epithelial Cells - JAK2-V617F Genotype (VAF%)
1-RK	GG (100%)	GG (100%)	GG (100%)
2-HB	GG (100%)	GG (100%)	GG (100%)
3-AT	**GG (100%)**	**GG / GC (G 99% G, C 1%)**	**GG (100%)**
4-MDA	GG (100%)	GG (100%)	GG (100%)
5-RÖ	GG (100%)	GG (100%)	GG (100%)
6-FS	GG (100%)	GG (100%)	GG (100%)
7-OO	GG (100%)	GG (100%)	GG (100%)
8-AK	**GG / GC (G 99,56%, C 0,43%)**	**GG / GC (G 98%, C 2%)**	**GG (100%)**
9-İK	**GG / GC (G 99%, C 1%)**	**GG / GC (G 97%, C 3%)**	**GG (100%)**
10-HE	GG (100%)	GG (100%)	GG (100%)