# COMPARATIVE ANALYSIS OF BLOOD VESSEL DENSITY IN UNCHANGED BONE MARROW, ACUTE AND CHRONIC MYELOID LEUKEMIA

## *KOMPARATIVNA ANALIZA GUSTINE KRVNIH SUDOVA U NEIZMENJENOJ KOŠTANOJ SRŽI, AKUTNOJ I HRONIČNOJ MIJELOIDNOJ LEUKEMIJI*

**David STANIĆ[1], Jelena ILIĆ SABO[2,4], Tanja LAKIĆ[3,4], Ivanka PERČIĆ[5,6], Marina DOKIĆ[5,6] and Nikola STOJŠIĆ[1]**

ORCID NUMBER
David Stanić – 0009-0008-4325-447X
Jelena Ilić Sabo – 0000-0002-5003-8512
Tanja Lakić – 0000-0003-4246-5132
Ivanka Perčić – 0000-0002-3462-5338
Marina Dokić – 0009-0009-4100-3666
Nikola Stojšić – 0009-0006-2819-3133

University of Novi Sad, Faculty of Medicine Novi Sad[1]
University of Novi Sad, Faculty of Medicine Novi Sad, Department of Histology and Embryology[2]
University of Novi Sad, Faculty of Medicine Novi Sad, Department of Pathology[3]
University Clinical Center of Vojvodina, Novi Sad, Center for Pathology and Histology[4]
University of Novi Sad, Faculty of Medicine Novi Sad, Department of Internal Medicine[5]
University Clinical Center of Vojvodina, Novi Sad, Clinic of Hematology[6]

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**Abstract**

**Introduction.** Acute myeloid leukemia and chronic myeloid leukemia are malignant blood cell disorders characterized by increased angiogenesis. This study aimed to evaluate blood vessel density in bone marrow tissue of patients with acute and chronic myeloid leukemia and to determine whether there is a statistically significant difference compared to a control group. **Material and Methods.** This retrospective study included 90 bone marrow biopsy samples, categorized into three groups: control, acute myeloid leukemia, and chronic myeloid leukemia. Pathohistological diagnoses were retrieved from the records of the Center for Pathology and Histology of the Clinical Center of Vojvodina. Samples were processed using standard histological and immunohistochemical techniques, examined microscopically, and photographed. Blood vessel density was quantified using ImageJ program, analyzing five microphotographs per sample, with results expressed as percentages. The mean value was calculated for each subject. Data were analyzed statistically and presented numerically, graphically, and in tables. **Results.** The mean blood vessel density was 1.85% (±0.56%) in the control group, 3.72 (±1.67%) in the acute myeloid leukemia group, and 5.42% (±2.28%) in the chronic myeloid leukemia group. A statistically significant difference in blood vessel density was observed among the groups. **Conclusion.** The statistically significant difference in blood vessel density between the groups underscores the role of angiogenesis in the pathogenesis of these malignancies and suggests its potential relevance in diagnosis and therapeutic strategies.

**Key words**: Blood Vessels; Angiogenesis; Bone Marrow; Leukemia, Myeloid; Biopsy; Morphological and Microscopic Findings

**Sažetak**

**Uvod.** Akutna mijeloidna leukemija i hronična mijeloidna leukemija su maligne bolesti krvnih ćelija koje karakteriše pojačana angiogeneza. Cilj ove studije bio je da se odredi gustina krvnih sudova u koštanoj srži kod pacijenata sa akutnom i hroničnom mijeloidnom leukemijom i da se ispita da li postoji statistički značajna razlika u poređenju sa kontrolnom grupom. **Materijal i metode.** Ova retrospektivna studija obuhvatila je 90 uzoraka biopsije koštane srži, podeljenih u tri grupe: kontrolnu grupu, grupu sa akutnom mijeloidnom leukemijom i grupu sa hroničnom mijeloidnom leukemijom. Patohistološke dijagnoze su dobijene iz dokumentacije Centra za patologiju i histologiju Univerzitetskog kliničkog centra Vojvodine. Uzorci su obrađeni standardnim histološkim i dodatnim imunohistohemijskim metodama, pregledani mikroskopski i fotografisani. Gustina krvnih sudova merena je pomoću programa *Image J*, analizom pet mikrofotografija po uzorku, a rezultati su izraženi u procentima. Za svakog ispitanika izračunata je prosečna vrednost. Podaci su statistički obrađeni i prikazani numerički, grafički i u tabelama. **Rezultati.** Prosečna gustina krvnih sudova u kontrolnoj grupi iznosila je 1,85% (± 0,56%), u grupi sa akutnom mijeloidnom leukemijom 3,72% (± 1,67%), dok je u grupi sa hroničnom mijeloidnom leukemijom iznosila 5,42% (± 2,28%). Utvrđena je statistički značajna razlika u gustini krvnih sudova između ispitivanih grupa. **Zaključak.** Utvrđena statistički značajna razlika u gustini krvnih sudova između grupa ukazuje na značaj angiogeneze u patogenezi ovih bolesti i potencijalnu ulogu ovog parametra u dijagnostici i terapiji.

**Ključne reči:** krvni sudovi; angiogeneza; koštana srž; mijeloidna leukemija; biopsija; morfološki i mikroskopski nalazi

## Introduction

Hematopoiesis - the process of generating blood elements in adulthood - takes place in the bone marrow, which is functionally divided into active red marrow and inactive yellow marrow composed primarily of adipocytes [1]. The active bone marrow houses hematopoietic stem cells, nutrient-carrying blood vessels, sinusoidal capillaries, and supportive tissue cells. Specific sinusoidal capillaries contain adventitial cells that secrete cytokines and factors essential for hematopoiesis [2–4].

All blood elements originate from a common pluripotent stem cell, which differentiates into lymphoid and myeloid lineages [2–4]. Disruptions of this process can lead to malignancies such as acute myeloid leukemia (AML). AML is marked by clonal prolifera-

✉ Corresponding author: Jelena Ilić Sabo, E-mail: jelena.ilic-sabo@mf.uns.ac.rs

***Abbreviations***

AML – acute myeloid leukemia
CML – chronic myeloid leukemia
VEGF – vascular endothelial growth factor
bFGF – basic fibroblast growth factor
CONTR – control group

tion of myeloid cells, accumulation of malignant blasts in the bone marrow, and suppression of normal hematopoiesis [5–8]. This malignant process often leads to bleeding, anemia, and infections in many patients. Diagnosis is based on pathohistological evaluation, with advancements in immunophenotyping (via flow cytometry) and cytogenetics. While the 2016 WHO classification defined AML by ≥20% blasts in the bone marrow, the 2022 revision introduced lower thresholds and incorporated new genetic parameters [8,9]. Auer rods may be present in myeloblast cytoplasm, and key cytochemical markers include myeloperoxidase and Sudan Black [10]. The five-year survival rate for AML remains below 30% [8].

Chronic myeloid leukemia (CML), another hematological malignancy, arises from disrupted differentiation of hematopoietic elements due to genetic translocation leading to the formation of the Philadelphia chromosome [11, 12]. CML progresses through three phases: the chronic phase (characterized by leukocytosis <10% blasts), the accelerated phase (with 10-19% blasts and marked basophilia), and the blast phase (defined by ≥20% blasts and extramedullary proliferation), which is the terminal stage indicating clinical progression of CML. The advent of tyrosine kinase inhibitors has dramatically improved outcomes, with a five-year survival rate now exceeding 90% [11].

Angiogenesis - the formation of new blood vessels from existing vasculature - is essential for tissue development and function, providing oxygen and nutrients to cells. However, in malignancies, angiogenesis facilitates tumor growth and dissemination, making it a critical factor in cancer progression. Key mediators of this process include vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF). While the role of angiogenesis in solid tumors is well-established, its involvement in the onset and progression of leukemias remains underexplored [12–17].

The aim of this study is to assess the percentage of blood vessel density in bone marrow samples from patients with AML and CML, and to determine whether statistically significant differences exist compared to healthy bone marrow.

## Material and Methods

This retrospective study was conducted using archival material obtained from the Center for Pathology and Histology at the University Clinical Center of Vojvodina, covering the period from January 2020 to December 2022. The study received ethical approval from the Ethics Committee of the University Clinical Center of Vojvodina (No. 00-257). A total of 90 bone marrow biopsy samples were included and categorized into three groups: a control group consisted of subjects with healthy bone marrow (CONTR), a group of patients diagnosed with acute myeloid leukemia (AML),a group of patients with chronic myeloid leukemia (CML), with 30 samples in each group.

Demographic data, including gender and age, were extracted from biopsy records. Based on age, subjects were stratifies into three groups: 1) younger than 46 years, 2) 46-65 years, and 3) over 65 years. The age threshold of 46 years was selected according to the study by Aljabban and Alalsaidissa, which identified this as the median age at AML diagnosis [18]. The group older than 65 years was of particular interest due to increased morbidity and reduced survival associated with treatment in this population [19]. Morphological analysis was carried out by the Center for Pathology and Histology in collaboration with the Clinic for Hematology. Bone marrow samples were processed using standardized procedures, including decalcification and full paraffin embedding. Tissue sections were stained with hematoxylin and eosin, Giemsa, and reticulin stains. Immunohistochemical analysis was also performed using CD34 antibody (Product**:** Monoclonal Mouse Anti-Human CD34 Class II, Clone: QBEnd-10, Catalog No.: M7165, Manufacturer: Dako/Agilent Technologies, Denmark).

Microscopic evaluation was conducted using a ZEISS Axioscope 5 light microscope at magnifications of 50x, 100x, 200x, 400x, and 630x. For each sample, five microphotographs were taken at 200x magnification using a ZEISS Axiocam 105 color camera. Image analysis was performed with ImageJ software to determine the areal density of blood vessels in the bone marrow, expressed as a percentage on each of the five images. The average blood vessel density value for each subject was then calculated.

Statistical analysis was conducted using IBM SPSS Statistics 26. The distribution of variables and the significance of differences in blood vessel density among the three study groups were assessed. Welch's ANOVA was used to evaluate statistical differences between independent parametric samples, followed by the Games-Howell post-hoc test for further analysis. The statistical significance level for these tests was set at p=0.05. Results are presented through microphotographs, numerical summaries, graphical displays, and tables.

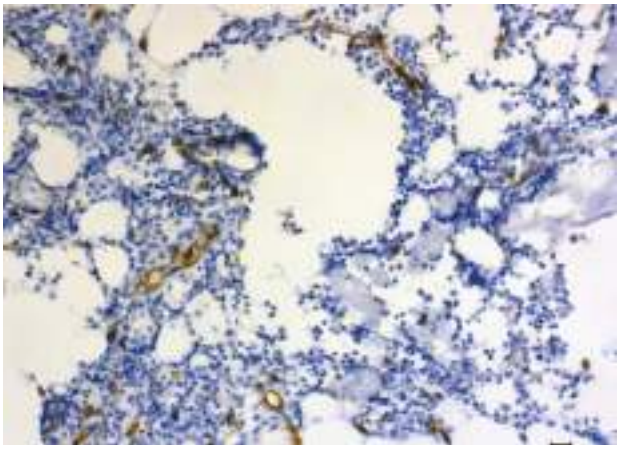

**Figure 1.** Blood vessels in participants with unchanged bone marrow, CD34, 200x

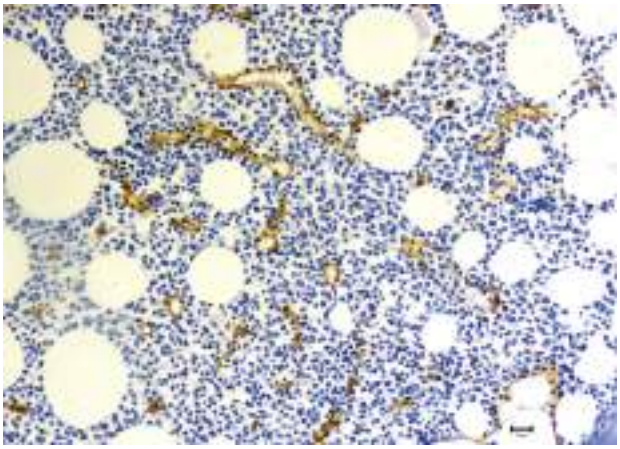

**Figure 2.** Blood vessels in patients with diagnosed AML, CD34, 200x

**Table 1.** Age of the participants in the observed groups

| Group | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|
| CONTR | 25 | 75 | 51 | 15.18 |
| AML | 29 | 89 | 58 | 13.5 |
| CML | 32 | 86 | 66 | 14.02 |

Legend: CONTR – control group; AML – acute myeloid leukemia; CML – chronic myeloid leukemia; SD – standard deviation

**Table 2.** Distribution of participants by age groups

| Observed group | Number and percentage of participants in each age group | | |
|---|---|---|---|
| | 0-45 years | 46-65 years | >65 years |
| CONTR | 11 (36.7%) | 11 (36.7%) | 8 (26.7%) |
| AML | 5 (16.7%) | 14 (46.7%) | 11 (36.7%) |
| CML | 4 (13.3%) | 8 (26.7%) | 18 (60%) |

Legend: CONTR – control group; AML – acute myeloid leukemia; CML – chronic myeloid leukemia

## Results

In the control group, participants ranged in age from 25 to 75 years, with a mean age of 51 ± 15.18 years. In the AML group, patient ages ranged from 29 to 89 years, with a mean age of 58 ± 13.5 years. Only 5 patients (16.7%) in this group were under 46 years of age. In the CML group, the youngest patient was 32 years old and the oldest was 86, with the mean age of 66 ± 14.02 years. A majority of patients in the CML group (17/30; 57%) were older than 65 years **(Tables 1 and 2).** In the control group, 14 participants (46.7%) were female and 16 (53.3%) were male. Among patients with AML, 17 (56.7%) were female and 13 (43.3%) male. In the CML group, there were slightly more male patients (17; 56.7%) compared to females (13; 43.3%). Statistical analysis revealed no significant differences in gender distribution between groups ($p > 0.05$).

Blood vessel density in unchanged bone marrow samples ranged from 0.87% to 2.84% per analyzed field, with an average density of 1.85 ± 0.56% **(Figure 1).** In

**Table 3.** Results of the Games-Howell Post-Hoc Test with the dependent variable "blood vessel density"

| (I) Group | (J) Group | Mean difference (I-J) | Standard error | p | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower bound | Upper bound |
| Control group | AML | -1.87* | .321 | .000 | -2.66 | -2.66 |
| | CML | -3.578* | .428 | .000 | -4.62 | -2.52 |
| Group with AML | CONTR | 1.87* | .32 | .000 | 2.66 | 2.66 |
| | CML | -1.7* | .52 | .023 | -2.94 | -.45 |
| Group with CML | CONTR | 3.57* | .43 | .000 | 2.52 | 4.62 |
| | AML | 1.7* | .52 | .023 | -.45 | 2.94 |

Legend: CONTR – control group; AML – acute myeloid leukemia; CML – chronic myeloid leukemia; p – significance

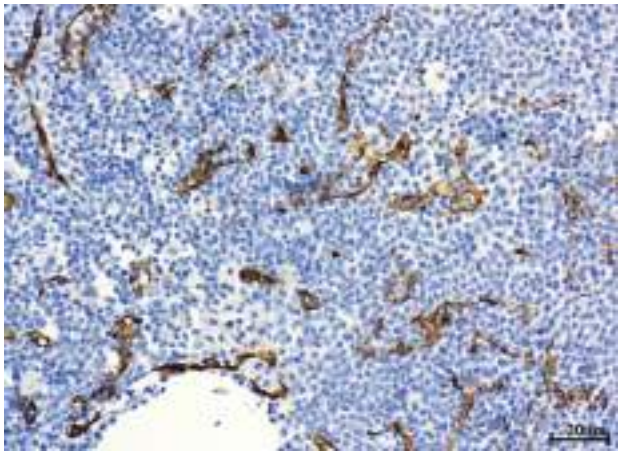

**Figure 3.** Blood vessels in patients with diagnosed CML, CD34, 200x

patients with AML, vessel density ranged from 1.33% to 7.1%, with a mean of 3.72 ± 1.67%) **(Figure 2),** indicating increases number of blood vessels in this condition.

The CML group exhibited the highest vascular density, ranging from 2.83% to 13.19%, with a mean of 5.42 ± 2.28% **(Figure 3)**. In 29 out of 30 CML cases (97%), the blood vessel density exceeded the highest value recorded in the control group.

Statistical analysis using Welch's ANOVA for independent samples within each predefined group demonstrated a statistically significant difference in blood vessel density among the three groups ($F_{2,44.364} = 47.724$, $p<0.05$). Post-hoc analysis using the Games-Howell test confirmed statistically significant differences in blood vessel density between all pairwise group comparisons: control vs. AML, control vs. CML, and AML vs. CML **(Table 3).**

## Discussion

In malignant hematologic neoplasms such as acute myeloid leukemia (AML) and chronic myeloid leukemia (CML), angiogenesis is markedly accelerated and intensified. This process is essential for tumor growth and progression, with its extent often correlating with the malignancy grade and potential dissemination. Our study analyzed this parameter and compared the results with previously published findings.

The average age of patients with AML in our study was 58 ± 13.5 years, aligning with previous literature that consistently reports a predominance of AML cases in individuals over 50 years of age [17,20–22]. In the CML group, the mean age was 66 ± 14.02 years, which slightly differs from other studies. For instance, Lopez-Hidalgo et al. reported the mean age of 49 years, while Hehlmann et al. documented an age range of 44 to 53 years [12,15,23].

The gender distribution in our study was relatively balanced. In the control group, 47% were female. In the AML group, 57% of the samples were from females, whereas in the CML group, 57% were from males. These findings are consistent with other studies that report no significant gender predominance in AML or CML, even in larger cohorts [12,15, 17,20,21,23,24].

Angiogenesis is well-documented as a key factor in the progression of solid tumors, facilitated by growth factors such as VEGF and bFGF. These molecules stimulate rapid formation of new blood vessels, supporting tumor survival and expansion. In solid tumors, an increased number of blood vessels is detected upon biopsy analysis, which puts emphasis on this process, and underscores the potential importance of analyzing this parameter in hematologic malignancies as well [13–17].

Our study demonstrated a statistically significant increase in bone marrow blood vessel density in patients with AML and CML compared to healthy controls. The average blood vessel density per field was 1.85 ± 0.56% in healthy individuals, 3.72 ± 1.67% in AML patients, and 5.42 ± 2.28% in CML patients. These findings are in line with those reported by Agauayo et al., who found blood vessel densities of 2.8% in healthy controls, 4.3% in AML, and 6.2% in CML patients [14]. Hussong et al. similarly observed twofold increase in bone marrow blood vessels in AML patients compared to controls [13]. Korkolopoulou et al., reported a threefold increase in CML [15], and Lundberg et al. observed twofold increase [25]. These and other studies confirm the heightened angiogenesis in these malignant neoplasms, which supports our findings [16,17,20,21].

Importantly, our results revealed a statistically significant difference in bone marrow blood vessel density not only between leukemia patients and healthy controls, but also between the AML and CML groups.

## Conclusion

Analysis of 90 bone marrow biopsies demonstrated that patients with acute myeloid leukemia were most frequently aged between 46 and 65 years, while those with chronic myeloid leukemia were predominantly over 65. Gender distribution was relatively balanced across all groups. Statistical analysis revealed a significantly increased bone marrow blood vessel density in both acute myeloid leukemia and chronic myeloid leukemia patients compared to the control group, with a significant difference in blood vessel density between acute myeloid leukemia and chronic myeloid leukemia patients.

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