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EDITORIAL

Dear Colleagues,

We dedicate this issue of our journal to the great commander Mustafa Kemal Atatürk and his comrades-in-arms, who achieve victory the Battle of the Commander-in-Chief, and to the loyal and great Turkish Nation, who spared no sacrifice at the cost of their lives and property. We remember all our veterans and martyrs with mercy. May their souls rest in peace….

İlhami BERBER. MD
Editor in Chief

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ORIGINAL ARTICLE

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CASE REPORT

Journal of Current Hematology & Oncology Research

Original Article

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Retrospective evaluation of our patients diagnosed with advanced pancreatic cancer

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ABSTRACT

Aims: Pancreatic cancer attracts attention with its increasing frequency among gastrointestinal cancers. It has the worst prognosis of all cancer types. In this study, we aimed to retrospectively evaluate the clinical features and treatment results of our patients with advanced pancreatic cancer.

Methods: In this study, 131 patients diagnosed with advanced pancreatic cancer and admitted to the Ankara Numune Training and Research Hospital Medical Oncology Polyclinic between November 2002 and January 2014 were retrospectively analyzed. Demographic characteristics of the patients, smoking, family history, basal CEA and basal CA 19-9 levels, treatment modalities, metastasis sites, progression-free survival, and overall survival times were evaluated.

Results: The study population consisted of 131 patients, including 94 males and 37 females. Twenty of the patients were in the locally advanced stage, and 111 were in the metastatic stage. The median age of the patients was 60 years. 53.4% of the patients were smokers, and the median amount of smoking was 30 packs/year. Adenocancer was the most common histopathological subtype. The median basal CEA and Ca 19-9 levels were high at the time of diagnosis. Neoadjuvant chemotherapy was given to all locally advanced patients. The most commonly used chemotherapy regimens were single-agent gemcitabine and gemcitabine-cisplatin combination therapies. While 10 of the locally advanced patients received local treatment (chemotherapy or surgery) after neoadjuvant chemotherapy, the remaining 10 patients could not receive local treatment. The median overall and progression-free survival times were found to be significantly longer in patients with locally advanced disease who received local treatment after neoadjuvant chemotherapy [(18 months vs. 6.5 months; p=0.008), (11.3 months vs. 6.4 months; p=0.05)]. The most common site of metastasis in our patients with metastatic pancreatic cancer was the liver (80%). Cisplatin-gemcitabine was preferred as a KT regimen in 63.4% of metastatic cases. The median overall survival time of the patients in the metastatic stage was 8.1 months, and the median progression-free survival time was 5.7 months. The median progression-free survival of all patients included in the study was 6.2 months, while the median overall survival was 8.8 months.

Conclusion: The median overall survival of patients with locally advanced pancreatic cancer was significantly longer compared to metastatic patients (18 months vs. 8.1 months; p=0.028). Progression-free survival and overall survival of patients with locally advanced disease who received or did not receive local treatment after neoadjuvant chemotherapy were found to be significantly longer in patients who received local treatment. (11.3 months vs. 6.4 months; p=0.05) (18 months vs. 6.5 months; p=0.008). Response rates of patients with metastatic pancreatic cancer; It was 49% in the gemcitabine-cisplatin combination arm and 30% in the single-agent gemcitabine arm.

Keywords: advanced pancreatic cancer, gemcitabine, cisplatin, survival

INTRODUCTION

Pancreatic cancer is one of the most common cancers in the world and is an important cause of cancer-related deaths.[1] It has attracted attention with its increasing frequency among gastrointestinal cancers in recent years.[2] At the time of diagnosis, the disease is usually at an advanced stage, and distant organ metastasis is encountered. Since there are no obvious symptoms in the early stages of the disease, the majority of patients lost the chance of curative resection at the time of diagnosis.[3] Palliative surgeries and systemic or regional treatments are usually applied to these patients who cannot be operated on.

According to the SEER (U.S. Surveillance, Epidemiology, and End Results) database, the 5-year survival rate for localized pancreatic cancer patients is 31.5%, 11.5% for those with regional disease, and 2-5% for patients with advanced metastases.[4] Factors affecting survival include age, gender,

comorbidities and habits. However, the most important factor affecting the outcome is the stage of the tumor at the time of diagnosis.[5] Survival in metastatic patients is around 3-6 months.[5]

The curative treatment of pancreatic cancer is surgical resection.[6] Most patients with pancreatic adenocarcinoma are metastatic at diagnosis. In patients with distant organ metastases and locoregional unresectable tumors, chemotherapy is the primary treatment option.[7]

In this study, we aimed to evaluate the clinicopathological features, prognostic factors, clinical course, treatment modalities, and overall and progression-free survival times of our patients with locally advanced and metastatic pancreatic cancer.

METHODS

Between November 2002 and January 2014, 131 patients with advanced pancreatic cancer (locally advanced and metastatic) who were admitted to the Medical Oncology Outpatient Clinic of Ankara Numune Training and Research Hospital were included in the study. Information about the patients was obtained through the retrospective examination of patient files.

Gender, age at diagnosis, smoking status and amount, family history of cancer, history and duration of diabetes mellitus, tumor stage, baseline CEA and CA 19-9 levels, treatment modalities, chemotherapy protocols, site of metastasis, chemotherapy response rates, last updated status, and last contact dates were recorded. Biopsy types, histopathologic types, and pathologic differentiation grades were analyzed from the patients' files. Progression-free and overall survival rates were determined. The study was approved by the Ankara Numune Training and Research Hospital's Ethics Committee.

RECIST (Response Evaluation Criteria in Solid Tumors) 1.1 criteria were used to evaluate the response to chemotherapy.[8] Complete response was defined as the disappearance of all target lesions; partial response as at least 30% reduction in the sum of the longest diameters of the target lesions; progression as at least 20% increase in the sum of the longest diameters of the target lesions; and stable response as no shrinkage as a partial response and no growth as progressive disease (<30% shrinkage, <20% growth).

Overall survival was calculated as the time from the date of diagnosis until death, and for patients who were still alive at the end of the study, the time from the date of the update of patient information was taken as the basis. Progression-free survival was calculated based on the time from diagnosis to progression. In patients who died before progression evaluation, the date of death was considered the date of progression.

The data obtained were interpreted by descriptive and comparative statistical analysis using the "SPSS for Windows 18" package program in a computer environment. In the study, variables with categorical values were given with numbers and percentages, and measurement variables with continuous values were given with median, minimum, and maximum values. The Kaplan-Meier method was used to construct survival curves, and the Log-rank test was used to compare variables in survival analysis. A value of $p<0.05$ was considered significant in statistical analyses.

RESULTS

Of the patients included in the study, 15% (n=20) were locally advanced and 85% (n=111) were metastatic. 71.8% (n=94) were male and 28.2% (n=37) were female. The male/female ratio was 2.54. The median age of the patients was 60 (28-80) years. The clinical and demographic characteristics of the patients are given in detail in **Table 1**.

Table 1. Clinical and demographic characteristics of the patients (n=131)

	n	%
Stage		
Locally advanced	20	15
Metastatic	111	85
Gender		
Male	94	71.8
Female	37	28.2
Age (years) (median-range)	60 (28-80)	
Cigarette consumption	70	53.4
Cigarette quantity (pack/year) (median-range)	30 (1-100)	
Diabetes mellitus	50	38.1
Diabetes mellitus duration (year) (median-range)	4 (1-15)	
Family history of cancer	27	20.6
Histopathological type		
--Adenocancer	100	76.3
--Malignant epithelial tumor	23	17.6
--Other *	8	6.1
Basal CEA ** (IU/L) (median-range)	6.6 (0-1440)	
Basal Ca 19-9 ***(IU/L) (median-range)	929 (1-120000)	

* Other: Adenosquamous (1), squamous cell (1), anaplastic carcinoma (1), carcinoma (4), neuroendocrine tumor (1)
**CEA: Carcinoembryonic antigen
***CA 19-9: Carbohydrate antigen 19-9

Of the 20 patients in the locally advanced stage, 11 were male and 9 were female. The male/female ratio was 1.22/1. The median age of the patients was 60 (42-78) years. When the patients were analyzed according to smoking characteristics, 35% were smokers, and 43% of them were active smokers at the time of diagnosis. The median amount of smoking was 25 (10-40) pack/year. When the patients were analyzed in terms of comorbid Diabetes Mellitus (DM), it was observed that 40% of the patients had a history of DM. Of the 8 patients with DM, 2 were newly diagnosed DM and 4 were using insulin. When the family history of cancer was investigated, 5 patients had a family history of cancer, but none of them had a family history of pancreatic cancer. When the types of biopsies performed during the pathologic diagnosis were evaluated, 80% of the patients were diagnosed with a tru-cut' biopsy. The histopathologic diagnosis was adenocarcinoma in 80% of the patients. When the baseline CEA and CA19-9 values of the patients were analyzed, the median baseline CEA level was 5 (1.6-72) IU/L and the median baseline CA 19-9 level was 355 (3.4-1200) IU/L. All patients received chemotherapy as neoadjuvant treatment. Of the 20 patients, 12 received cisplatin-gemcitabine combination chemotherapy and 8 received single-agent gemcitabine. Regarding the response rates after chemotherapy, 12 (60%) patients had a stable response, 4 (20%) had a partial response, and 4 (20%) had progression. Clinical and pathologic characteristics of the patients with locally advanced pancreatic cancer are given in **Table 2**.

Table 2. Clinical and pathological features of locally advanced patients (n=20)	
	n (%)
Age (years) (median-range)	60 (42-78)
Gender	
Male	11 (55)
Female	9 45)
Male/Female	1.22
Cigarette consumption	7 (35)
Cigarette quantity (pack/year) (median-range)	25 (10-40)
History of DM	8 (40)
Family history of cancer	5 (25)
Biopsy types	
Tru-cut biopsy	16 (80)
EUS-FNAB	3 (15)
ERCP-brush biopsy	1 (5)
Histopathological types	
Adenocancer	16 (80)
Malignant epithelial tumor	3 (15)
Undifferentiated carcinoma	1 (5)
Differentiation	
Grade 1	2 (10)
Grade 2	1 (5)
Grade 3	1 (5)
Unknown	16 (80)
Basal CEA (IU/L) (median-range)	5 (1.6-72)
Basal CA 19-9 (IU/L) (median-range)	355 (3.4-1200)
CTX Regimes	
Gemcitabine	8 (40)
Gemcitabine-Cisplatin	12 (60)
Treatment response	
Stable response	12 (60)
Partial response	4 (20)
Progression	4 (20)

Of the 20 patients with locally advanced disease who received neoadjuvant chemotherapy (CT), 10 received local treatment (surgery and chemoradiotherapy) after neoadjuvant CT. While 9 of these patients received chemoradiotherapy as local treatment, one of them underwent surgery. The overall survival time of the operating patient was 11.7 months, and the progression-free survival time was 11.3 months. Only one of the 10 patients who could receive local treatment died. That patient had received chemoradiotherapy as a local treatment. Of the remaining 10 patients who did not receive local treatment, 3 died due to disease progression. When the median overall and progression-free survival times of patients with locally advanced disease who received local treatment after neoadjuvant KT and those who did not were compared, they were significantly longer [(18 months vs. 6.5 months; p:0.008), (11.3 months vs. 6.4 months; p:0.05)]. The median overall survival time of all patients with locally advanced pancreatic cancer was 18 months, and the progression-free survival time was 8.7 months.

Of the 131 patients with advanced pancreatic cancer, 111 were metastatic at the time of diagnosis. Of the patients, 83 (74.8%) were male and 28 (25.2%) were female. The male/female ratio was 2.96/1. The median age of the patients was 60 (28-80) years. When the patients were analyzed according to smoking characteristics, 63 (56.7%) had a history of smoking, and 57% were active smokers at the time of diagnosis. The median value of the amount of smoking was 30 (1-100) pack/year. In terms of DM, 42 patients (38%) had a history of DM. The diagnosis of DM was new in 8 of the patients with DM, and 22 of them had a history of insulin use and 8 of them had a history of metformin use. In terms of family history of cancer, 23 (20.7%) patients had a family history of cancer, and 3 of the 23 patients had a family history of pancreatic cancer, unlike patients with locally advanced pancreatic cancer. When the histopathologic diagnosis was analyzed, 84% were diagnosed as adenocarcinoma. Most of the patients whose differentiation was specified in the pathology notes in the patient files were in grade 3. When the baseline tumor markers of the patients were analyzed, the median baseline CEA level was 7.9 (0.2-1140) IU/L and the median baseline Ca 19-9 level was 1000 (0.6- 120000) IU/L. Clinical and pathologic characteristics of the patients with metastatic pancreatic cancer are given in **Table 3**.

Table 3. Clinical and pathological features of metastatic patients (n=111)	
	n (%)
Age (years) (median-range)	60 (28-80)
Gender	
Male	83 (74.8)
Female	28 (25.2)
Male/Female	2.96
Cigarette consumption	63 (56.7)
Cigarette quantity (pack/year) (median-range)	30 (1-100)
History of DM	42 (38)
Family history of cancer	23 (20.7)
Biopsy types	
Tru-cut biopsy	102 (91.9)
EUS-FNAB	4 (3.6)
ERCP-brush biopsy	3 (2.7)
Unknown	2 (1.8)
Histopathological types	
Adenocancer	84 (%75.7)
Malignant epithelial tumor	20 (%18)
Others*	7 (%6.3)
Differentiation	
Grade 1	1 (1)
Grade 2	11 (10)
Grade 3	24 (22)
Unknown	75 (67)
Basal CEA (IU/L) (median-range)	7.9 (0.2-1140)
Basal CA 19-9 (IU/L) (median-range)	1000 (0.6-120000)

* Others: Adenosquamous carcinoma (1), Anaplastic carcinoma (1), Carcinoma metastasis (3), Neuroendocrine tumor (1), Squamous cell cancer (1)

The most common site of first metastasis in patients with metastatic pancreatic cancer was the liver (80%), followed by the lung and the liver (11%). First-line CT was given to 93 metastatic patients. The gemcitabine-cisplatin combination was the most preferred regimen (63.4%). The second-most preferred regimen was single-agent gemcitabine (32.2%). The stable response rate was 44%, the progression rate was 34%, the partial response rate was 20.3%, and the complete response rate was 1.7%. The median overall survival time of patients with metastatic cancer was 8.1 months, while the median progression-free survival time was 5.7 months.

The median progression-free survival of patients with advanced pancreatic cancer (locally advanced and metastatic) included in our study was 6.2 months (95% CI: 5.4-6.9) and the median overall survival was 8.8 months (95% CI: 6.3-11.2). Progression-free and overall survival curves of patients with advanced (locally advanced and metastatic) pancreatic cancer are shown in **Figures 1 and 2**.

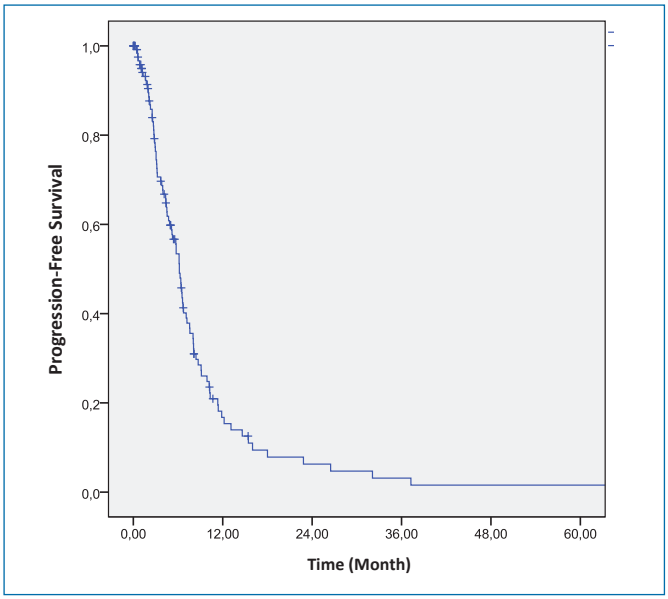

Figure 1. Progression-free survival of patients with advanced (locally advanced and metastatic) pancreatic cancer

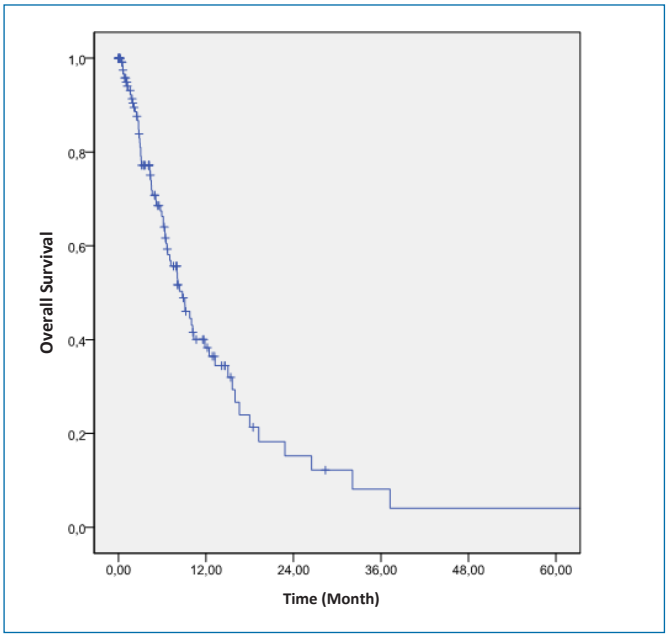

Figure 2. Overall survival of patients with advanced (locally advanced and metastatic) pancreatic cancer

DISCUSSION

Pancreatic cancer is most common in the 7th and 8th decades, and the median age at diagnosis is 60-65 years.[9] It has been reported that the risk of pancreatic cancer increases 2-3 times every decade after the age of 40 years.[10] The median age of the patients in our study was 60 years, similar to the data in the literature. The youngest patient was 28 years old at the time of diagnosis. The incidence of pancreatic cancer in men is higher than in women.[11] In recent years, the relative risk for women has been approaching that of men. The reason for this increase is thought to be the increase in smoking among women.[12] In our study, the male/female ratio was 2.54/1, and the incidence of pancreatic cancer was higher in men. Approximately 85-90% of pancreatic cancers are ductal adenocarcinoma.[13] 76.4% of our patients had an adenocarcinoma histopathologic subtype.

Seventy patients (53.4%) had a smoking history, and the median cigarette consumption was 30 pack/year. It is estimated that smoking contributes to the development of 20-30% of pancreatic cancers.[14] The incidence of pancreatic cancer is 75% higher in smokers. It has been reported that each pack/year of cigarettes consumed increases the risk of developing pancreatic cancer by 2%. As the number of cigarettes smoked increases, the risk of pancreatic cancer increases in parallel .[15]

It has been reported that high insulin concentrations, glucose intolerance, and insulin resistance may play a role in carcinogenesis.[16] Patients with DM for at least 5 years have a 2-fold increased risk of developing pancreatic cancer.[17] 50 (38.4%) of the patients in our study had comorbid DM. The median duration of DM was calculated at 4 years.

In terms of family history of cancer, 20.6% had a family history of cancer. It was observed that 11% of these patients had a family history of pancreatic cancer. All patients with a family history of pancreatic cancer had metastatic pancreatic cancer at the time of diagnosis. A history of pancreatic cancer in one first-degree relative increases the risk of pancreatic cancer 4.5 times; in 2 relatives, the risk increases 8.4 times; and in 3 relatives, the risk increases 32 times.[18] In patients with familial pancreatic cancer, the risk of pancreatic cancer increases, as well as the risk of non-pancreatic cancers (especially breast, ovarian, and biliary tract cancers).[19]

Similar to the literature, the most common metastatic site in our metastatic patients was the liver (80%). The second-most common metastasis was simultaneous liver and lung metastasis. When basal CA 19-9, one of the tumor markers, was analyzed, the median value was 929 IU/ml. It has been reported that pancreatic cancer patients presenting with high basal CA 19-9 levels have a worse prognosis than others.[20] Ni et al. found that high serum CA 19-9 levels were associated with distant metastasis, advanced stage, and a poor prognosis for pancreatic cancer.[21] When the median CA 19-9 levels of our locally advanced and metastatic patients at the time of diagnosis were compared, the CA 19-9 levels of metastatic patients were higher than those of locally advanced patients. In our study, the median CEA level of patients with advanced pancreatic cancer was 6.6 IU/ml, and our results were consistent with the literature.

In a study by Tsavaris et al., it was found that the CEA value increased with increasing tumor mass and was associated with a poor prognosis. In this study, those with a CEA value above 5 IU/ml had a 1.4-fold higher risk of death.[22] In a study by Trikudanathan G et al. in patients with pancreatic cancer, 70% had elevated CEA and CA 19-9 .[23]

In the treatment of pancreatic cancer, a triple treatment modality (surgery, RT, and CT) is preferred. The only potentially curative treatment modality in pancreatic cancer is surgery in early-stage disease.[24] In our study, all 20 patients with locally advanced disease received neoadjuvant CT. The preferred combination was gemcitabine-cisplatin (60%) and single-agent gemcitabine (40%). After neoadjuvant CT, 10 patients were able to receive local treatment (CRT or surgery). The median overall survival time and median progression-free survival time of the 10 patients with locally advanced disease who could receive local treatment (CRT or surgery) after neoadjuvant CT (18 months vs. 6.5 months, p:0.008) and the 10 patients who could not receive local treatment (11.3 months vs. 6.4 months, p:0.05) were significantly longer in the locally treated group. The fact that local treatment after neoadjuvant KT increased survival is promising for a cancer with such a poor prognosis.

Invasion of vascular structures in locally advanced pancreatic cancers makes surgical resection difficult. When the chemoradiotherapy outcomes of 323 patients with locally advanced pancreatic cancer at M.D. Anderson Cancer Center

were analyzed, 76 patients who received gemcitabine for 2.5 months before chemoradiotherapy had a longer median overall survival and progression-free survival.[25] In another retrospective series, 181 patients with locally advanced pancreatic cancer were given gemcitabine-containing chemotherapy for 3 months; 72 of the 128 patients who did not progress were continued with chemoradiotherapy, and the remaining 56 received chemotherapy. The median progression-free and overall survival times of patients who continued with chemoradiotherapy (10.8 months and 15 months) were longer than those of patients who continued with chemotherapy (7.4 months and 11.7 months) .[26] Systemic palliative chemotherapy for metastatic pancreatic cancer is primarily aimed at relieving disease-related symptoms.[27] 93 of the 111 metastatic patients included in the study could receive CT. The gemcitabine-Cisplatin combination (63.4%) and single-agent gemcitabine (32.2%) were the most preferred CT regimens.

In studies comparing the efficacy of gemcitabine and cisplatin combination with single agent gemcitabine in the treatment of metastatic pancreatic cancer, the response rate (26% vs. 9%) and median survival time (30 weeks vs. 20 weeks) were reported to be better in the combination arm.[28] In our study, the response rates of metastatic patients after first-line KT were 49% in the combination arm and 30% in the single-agent gemcitabine arm. In a multicenter retrospective study conducted in our country, the efficacy of cisplatin and gemcitabine combination and single agent gemcitabine in locally advanced or metastatic pancreatic cancer was compared, and it was found that the response rate was significantly higher in the combination arm (69% vs. 49.7%), but there was no significant difference between the treatment arms in terms of time to progression (8.9 months vs. 6 months) and overall survival (12 vs. 10.2 months).[29]

CONCLUSION

The results of our study clearly demonstrate the need for new chemotherapy regimens in the treatment of advanced pancreatic cancer. Pancreatic cancer is one of the cancer types with a very poor prognosis. The median time to progression of 6.2 months and median overall survival of 8.8 months in the patients in our study indicate the mortal course of the disease. When patients with locally advanced and metastatic pancreatic cancer were compared in terms of median overall and progression-free survival, median overall survival was longer in patients with locally advanced disease (p:0.028). Although all our patients had advanced disease, survival outcomes were worse in patients with systemic metastases.

ETHICAL DECLARATIONS

Ethics Committee Approval: This thesis research was authorized in 2010 by the institution of Dicle University. **Informed Consent:** Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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ß-catenin expression in myelodysplastic syndromes and myeloproliferative neoplasms in bone marrow, in relation to cd34 and cd117 status

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ABSTRACT

Aims: The activation of the Wnt/ β-catenin signaling pathway has been demonstrated to play a crucial role in the development of myeloid neoplasms. In addition to CD34, which has been used until now in the diagnosis and staging of clonal hematopoietic diseases, Myelodysplastic Syndromes (MDS) and Myeloproliferative Neoplasms (MPN), CD117, which has found its place in hematopoietic diseases, also provides significant benefits in these respects. In this study, we evaluated the immunohistochemical presence and utility of β-catenin in blasts relative to other markers, as the inhibition of β-catenin activity may be an attractive therapeutic approach

Methods: By retrospectively analyzing bone marrow samples with β-catenin immune marker, we determined the staining rates, intensities, and patterns of 30 MDS, 29 MPN cases and 30 normal bone marrow controls, in comparison to the efficacy of the the well-known CD34 and CD117. We statistically interpreted the correlation between them.

Results: Based on the findings, β-catenin, which has recently been used in hematopoietic diseases and is said to have a high efficacy in acute myeloid leukemia (AML) cases, was not immunohistochemically detectable in our study. As expected, CD34 and CD117 immun markers exhibited significant blast staining. MPN cases were more prone to staining with CD117.

Conclusion: CD34 continues to be the most reliable marker for identifying blasts for diagnosing and grading bone marrow neoplasms while CD117 may have a supportive role in this process. Further investigation is required to ascertain the true effectiveness of β-catenin, a molecule that has demonstrated encouraging potential in the context of AML.

Keywords: Myelodysplastic syndrome, myeloproliferative neoplasm, β-catenin, CD34, CD117

INTRODUCTION

Both Myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) are classified as clonal hematopoietic disorders. There are distinct differences between MPN and MDS, starting from the earliest presentation of the disease. Genetic damage results in the selective proliferation of hematopoietic stem cells during late myeloid differentiation.[1] Deletion of chromosome 5q [del (5q)] is one of the most frequently observed cytogenetic abnormalities in patients with de novo myelodysplastic syndromes and therapy-related MDS or acute myeloid leukemia (t-MDS/t-AML).[2] Patients with del (5q) MDS have a favorable prognosis and a low risk of transformation to AML.[3] MDS frequently evolves into acute myeloid leukemia or bone marrow failure but years are required for the development of ineffective hematopoiesis and the appearance of blast forms in bone marrow and peripheral blood; its progression is typically synonymous with the onset of acute leukemia.[4,5,6] It might be argued that the identification of blast count represents a crucial stage in the course of AML.

CD34 is a glycophosphoprotein cell surface antigen expressed on progenitor and early precursor bone marrow cells that is very useful in identifying the presence of 'atypical localization of immature precursors' (ALIP), a characteristic finding in MDS. With increasing maturation, CD34 expression decreases, and weak positivity or negativity is observed in the final progenitor cells.[7] An increase in the number of CD34 (+) cells and their tendency to form aggregates are indicators of acute leukemic transformation.

The c-kit protooncogene encodes a receptor tyrosine kinase that serves as a ligand for the stem cell factor. This gene is expressed in several non-hematopoietic tissues as well as malignancies.[8] C-kit receptor (CD117) was found to be expressed in multipotent hematopoietic stem cells, myeloid and/or erythroid progenitors, and B and T lymphocyte progenitors.[9] CD117 expression is at its highest level in progenitor cells at the earliest phases of hematological

development, while it decreases as maturation advances. The highest levels of CD117 expression are observed in advanced-stage MDS and MPN cases in association with an increased percentage of blasts.[8]

The Wnt/β-catenin pathway is an signaling pathway that plays a crucial role in a variety of cell biology processes including gene expression, growth, proliferation, migration and adhesion.[10-12] Dysregulation of Wnt/β-catenin has been associated with tumorigenesis.[13-15] Researches elucidate the critical function of Wnt/β-catenin pathway activation in the development of myeloid neoplasms2. The objective of this study was to examine the immunostaining properties of β-catenin in comparison to CD34 and CD117, with the intention of identifying its potential as a reliable marker for recognizing blasts, which is essential for the diagnosis of myeloid neoplasms.

METHODS

This thesis research was authorized in 2010 by the institution of Dicle University. However, ethics committee approval was not required at that time. All procedures were carried out in accordance with the ethical rules and the principles of Helsinki Declaration of 1964, as revised in 2000. A total of 89 bone marrow materials and bone marrow smears, including 30 samples with myelodysplastic syndrome, 29 with myeloproliferative neoplasms, and 30 normocellular bone marrow controls without any cytological atypia that never diagnosed with a hematological disorder, were examined retrospectively in accordance with the WHO criteria. Cases were eligible if they were diagnosed with MDS and MPN, regardless of subtypes and grades. The bone marrow smears were stained with Giemsa. For the immunohistochemical examination, three paraffin block cross-sections were obtained and examined with β-catenin, CD34, and CD117 immunostains.

The cases were evaluated in four categories according to the rate of staining as follows: (i) cases without bone marrow staining or with a staining rate below 5%; (ii) cases with a staining rate between 5-30%; (iii) cases with a staining rate between 31-60%; and (iv) cases with a staining rate over 61%. In the assessment based on the intensity of staining with the immunostains, cases were categorized as follows: mild-to-minimum staining as 1(+), moderate staining as 2(+) and strong staining as 3(+). The cases were categorized into four groups based on the pattern of staining: membranous, cytoplasmic, membranous/cytoplasmic, and nuclear.

Statistical Evaluation

Statistical analyses were conducted with statistical package software Epi Info, version 2000 (CDC Atlanta). Chi-square test was used for the comparison of rates between the groups (with cross tabulations). In addition, ANOVA and post hoc, Tukey HSD, Kruskal-Wallis analysis of variance and t-test were used. Intergroup differences were considered statistically significant if the p-value was less than 0.05.

RESULTS

A total of 89 patients (30 MDS, 29 MPN and 30 control group) were examined in this study. Overall, 42 patients were male (47.2%) and 47 patients were female (52.8%). The mean age was 58.5 years (range, 21-79 years) for the MDS patients, 57.2 years (range, 18-79 years) for the MPN patients and 47.3 years (range, 20-82 years) for the control group. MDS patients had a significantly higher mean age compared to the patients with a normal bone marrow (p=0.03). The mean cellularity was 70.16% (35-95%) for MDS cases, 80.44% (25-75%) for MPN cases and 48.66% (25-75%) for the control group. Based on the statistical data, MPN cases demonstrated a significantly higher bone marrow cellularity (p<0.01).

Comparison of CD34 staining intensity between groups revealed a statistically significant difference (p<0.01)(**Table 1**).

Table 1. CD34 expression results in MDS, MPN and control groups

CD34 staining rates	Control Group		MDS Group		MPN Group		Total	
<%5	25	%83.3	9	%30	12	%41.4	46	%51.7
%5-30	5	%16.7	21	%70	17	%58.6	43	%48.3
>%30	0	0	0	0	0	0	0	0
CD34 staining intensity								
1(+)	5	%16.7	4	%13.3	0	0	9	%10.1
2(+)	15	%50	10	%33.3	10	%34.5	35	%39.3
3(+)	10	33.3	16	%53.3	19	%66.5	45	%50.6
CD34 staining pattern								
Cytoplasmic	29	%96.7	23	%76.7	28	%96.6	80	%89.9
Membranous /cytoplasmic	1	%3.3	7	%23.3	1	%3.4	9	%10.1
Total	**30**	**%100**	**30**	**%100**	**29**	**%100**	**89**	**%100**

MDS: Myelodysplastic Syndrome, MPN: Myeloproliferative Neoplasm

Compared to bone marrow controls, there was a high rate of CD34 staining in both MDS and MPN cases, as determined by paired comparisons (**Figure 1**). The vast majority (83.3%) of control cases exhibited a staining rate of less than 5%, whereas 70% of MDS cases and 58% of MPN cases exhibited staining rates of 5% or higher

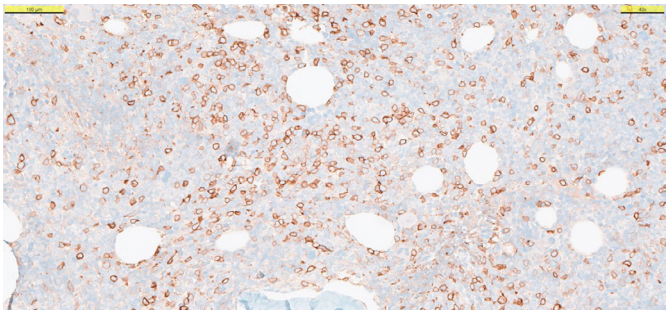

Figure 1. Memranous/stoplasmic CD34 expression in a myelodysplastic syndrome case (x400)

Although no significant differences were observed among all three groups with respect to CD34 staining intensity (p=0.06), a significant difference was observed in terms of CD34 staining pattern (p=0.01). Nuclear staining or membranous staining alone was not observed in any of the cases. The paired comparisons revealed that particularly the membranous/cytoplasmic staining pattern with CD34 was significantly higher in MDS cases when compared to the other two groups.

A significant difference was observed between MDS, MPN and control groups in terms of CD117 staining rate (p<0.01). In the vast majority (90%) of the control group, a staining rate less than 5% was observed for CD117, while staining rates equal to 5% and higher were found in 73.3% of the MDS cases and in 82.7% of the MPN cases (**Table 2, Figure 2**).

None of the cases with CD117 staining showed only membranous or nuclear staining. Regarding the CD117 staining pattern, no significant differences were observed between the groups (p=0.90).

Table 2. CD117 expression results in MDS, MPN and control groups

CD117 Staining rates	Control group		MDS Group		MPN Group		Total	
<%5	27	%90	8	%26.7	5	%17.2	40	%44.9
%5-30	3	%10	18	%60	24	%82.8	45	%50.6
>%30	0	0	4	%13.3	0	0	4	%4.5
CD117 Staining intensity								
1(+)	11	%36.7	7	%23.3	1	%3.4	19	%21.3
2(+)	14	%46.7	12	%40	15	%51.7	41	%46.1
3(+)	5	%16.7	11	%36.7	13	%44.8	29	%32.6
CD117 Staining pattern								
Cytoplasmic	25	%83.3	26	%86.7	24	%82.8	75	%84.3
Membranous /cytoplasmic	5	%16.6	4	%13.3	5	%17.2	14	%15.7
Total	30	%100	30	%100	29	%100	89	%100

MDS: Myelodysplastic Syndrome, MPN: Myeloproliferative Neoplasm

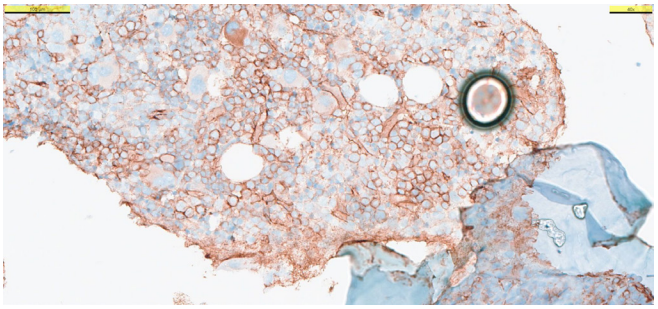

Figure 2. Memranous/stoplasmic CD117 expression in a myeloproliferative neoplasm case (x400)

When compared to the control group, both MDS and MPN groups had statistically substantially higher frequencies of β-catenin staining (p<0.01) (**Figure 3**).

No significant difference was observed among all three groups in terms of β-catenin staining intensity (p=0.32). Level 1(+) staining with β-catenin was observed in 86.5% of all cases, while level 2(+) staining was found in 13.4% of the cases. Cytoplasmic staining was observed in 96.6% of all cases (**Table 3**).

Examining the correlation between the expression rates of all three markers, a potent correlation (0.50-0.7-5) was observed between CD34 and CD117, as well as CD117 and β-catenin, in both MDS and MPN cases. A weak correlation (0.25-0.50) was observed between CD34 and β-catenin. In the control group, correlations between CD34 and CD117 were weak (0.25-0.50), while correlations between CD34 and β-catenin were minimal (0.25) and correlations between CD117 and β-catenin were strong (0.50-0.75).

Table 3. β-catenin expression results in MDS, MPN and control groups.

β-catenin staining rates	Control Group		MDS Group		MPN Group		Total	
<%5	30	%100	10	%33.3	17	%58.6	57	%64
%5-30	0	0	20	%66.6	12	%41.4	32	%36
>%30	0	0	0	0	0	0	0	0
β-catenin staining intensity								
1(+)	24	%80	28	%93.3	25	%86.2	77	%86.5
2(+)	6	%20	2	%6.7	4	%13.7	12	%13.4
3(+)	0	0	0	0	0	0	0	0
B-catenin staining pattern								
Cytoplasmic	29	%96.7	28	%93.3	29	%100	86	%96.6
Membranous /cytoplasmic	1	%3.3	2	%6.6	0	0	3	%3.3
Total	30	%100	30	%100	29	%100	89	%100

MDS: Myelodysplastic Syndrome, MPN: Myeloproliferative Neoplasm

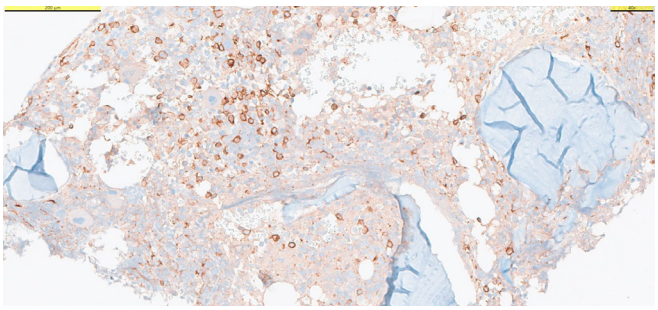

Figure 3. Memranous/stoplasmic β-catenin expression in a group of 'atypical localization of immature precursors' (ALIP) in a myelodysplastic syndrome case (x400)

DISCUSSION

MPN, first described by Dameshak in 1951, are clonal diseases of pluripotent hematopoietic stem cells characterized by neoplastic proliferation at least in one series in bone marrow. Due to clonal expansion, hematopoietic series exhibit an abnormally high level of proliferation and hematopoiesis. In contrast to acute leukemias, the maturation of cells is complete and the natural course of the disease is chronic in MPN.

MDS is a group of clonal stem cell diseases characterized by ineffective and dysplastic hematopoiesis and has a high risk for progression to acute leukemia. The most significant prognostic indicator for MDS is high blast count in blood and bone marrow.[5] Blast count is essential for the diagnosis and subtyping of numerous clonal hematopoietic diseases.

In a large number of studies, many surface antigens were tested for marking progenitor cells.[16,17] CD34 was one of the most investigated surface antigens that was found to be uniquely expressed on stem cells in studies.[18] It was observed to be significantly elevated, particularly in cases of high-grade MDS, which demonstrates an increased ALIP clusters and count of blast cells. In accordance with this, in this

study we observed substantially higher staining in MDS and MPN cases compared to the control group.

Furthermore CD117 was demonstrated to be more dependable and superior than various surface antigens. Although CD117 may be expressed in numerous non-hematopoietic tissues and in all bone marrow cell types, it has demonstrated a high level of specificity for the myeloid cell types in particular. Muroi et al. reported that CD117 is the most trustworthy marker for identifying myeloblasts.[19] In hematological diseases progressing with megakaryocytic and erythroid involvement, the expression of this gene was found to be much lower or even absent.[8] Di Noto et al. identified CD117 positivity in 62% of AML cases, in 64.7% of chronic myeloid leukemia patients with myeloid blast crises and in 92.8% of advanced MDS cases.[20] Nomdede'u JF et al. and Pirruccello et al. discovered CD117 expression in MDS blast cells at a rate of 91.6% and 57.2%, respectively.[18-21] Similar to the CD34 staining rates, the CD117 staining rates of the cases in our study were substantially distinct from those of the control cases when compared to the MDS and MPN groups. Furthermore the staining frequencies for CD34 and CD117 increase with increasing grade and blast count.

Although there is no statistically significant difference between MPN and MDS in terms of CD34 and CD117 staining rates, MPN cases tend to exhibit CD117 staining rather than CD34 staining. Since CD117 is primarily myeloid-specific, the higher CD117 staining rates observed in MPN cases are to be expected. Due to the prevalence of myeloid proliferation in MPN, the increase in granulocyte/macrophage series progenitors is substantially greater than that of other progenitors.

β-catenin is a cytoplasmic protein that serves as a component of the Wnt signaling pathway. Numerous studies have demonstrated a substantial elevation in the expression of β-catenin in patients of AML22. While β-catenin has been observed in several hematological disorders, its presence in MDS and MPN has only been shown in a limited number of studies.[23] For example, in the study of Jauregui et al., staining rates of more than 5% were observed in 17.3% of 52 MPN cases. In all six of the control cases, there was a staining rate of less than 5%24.

In this study, our results indicate that there are notable disparities in staining rates between the MDS and MPN groups when compared to the control group. 66.7% of the MDS cases and 41.4% of the MPN cases exhibited a staining rate of 5% or higher. The tendency of the MDS and MPN cases to exhibit a higher rate of β-catenin staining than the control cases may provide evidence for the role of the proliferation of hematopoietic series and progenitor cells in both diseases and the role of the Wnt signaling system in this proliferation. Consistent with the results of the study in the literature, all control cases had a staining rate of less than 5%. In our study, when considering MPN cases, higher β-catenin staining rates were observed compared to previous researches. The features of the patients as well as technical factors might be to blame for the variation in staining rates between the studies.

No significant differences were observed between all three groups with respect to the intensity of staining for β-catenin. Overall, 86.5% of the cases showed level 1(+) staining while no cases had level 3(+) staining.

In their study, Jauregui et al. observed that β-catenin staining was cytoplasmic in megakaryocytes and perinuclear/cytoplasmic in erythroid/myeloid series.[24] In some earlier studies, β-catenin was found to be expressed in both cytoplasmic and nuclear localizations in non-hematological neoplasms. In our study we have also observed cytoplasmic staining in 96.6% of the cases. There was no evidence of nuclear staining in any of the cases. Likewise, Subotiki T. et al. found no nuclear staining of β-catenin in MDS cases in their research. All cases displayed membranous and/or stoplasmic β-catenin staining, and patients with Polistemia Vera had the maximum number of immunexpressing cells.[23]

Jinglan Xu et al. observed nuclear β-catenin staining in myeloblasts and erythroblasts from patients with AML and MDS, noting that nuclear staining indicates that β-catenin is not depleted, is present, and is activated by being transported into the nucleus. They discovered nuclear staining in 40.9% of cases and reported that nuclear β-catenin staining is indicator of poor prognosis in both AML and MDS cases. Nuclear β-catenin staining was observed particularly in cases with high-grade MDS. This study presents a unique investigation of the presence of β-catenin staining in MDS25. The nuclear β-catenin staining is direct evidence for β-catenin activation, which demonstrates the importance of the Wnt/ β-catenin signaling system and that β-catenin activation is directly related to the increase in the number of blast cells in MDS. In our study, in MDS cases, cytoplasmic β-catenin staining was found in erythroid and endothelial cells. The observation of cytoplasmic β-catenin staining in normal erythroblasts suggests that cytoplasmic staining does not necessarily indicate β-catenin activation and that β-catenin exists ordinarily as an adhesion molecule on the inner surface of cell membranes. In support of this data, cases with normal bone marrow, Jinglan Xu et al. also had expression in the cell membranes and cytoplasm.[25]

When evaluating only the MDS cases in our study, CD34 shows superiority to CD117 and β-catenin in terms of staining rate. It is evident that progenitor cells and blasts have a high propensity to stain with CD34. Despite the fact that numerous immune indicators are used to determine an increased blast count, CD34 remains the most reliable marker in this regard. In our study of MPN cases, CD117 staining rates were substantially higher than those of the other two markers. CD117 is a marker which mainly shows a myeloid specificity and is expressed at higher levels in granulocyte/myeloid progenitor cells rather than in primitive hematopoietic progenitors. Therefore, it is expected to stain at high levels in MPN cases, particularly with proliferation in myeloid series. Interestingly, β-catenin staining rates were higher in the control group of our study when compared to the other two groups. β-catenin is an adhesion protein that can also normally be found in the inner cell membrane. It may exist in the cytoplasm even in the absence of any pathology. On the other hand, CD34 and CD117 antigens manifest themselves mainly in the neoplastic proliferation of blasts and progenitor cells.

In our study, we observed a good level of correlation between CD34 and CD117 as well as between CD117 and β-catenin in both MDS and MPN cases, while a weak correlation was found between CD34 and β-catenin. In their study, Ysebaert et al. reported a correlation between β-catenin and CD34 expression. In our study, this correlation was weak.[26]

Limitations of the study

This study had both some quantitative and methodological limitations. The main challenge was studying immunohistochemical techniques without any antigen loss in long-standing, acid-treated bone marrow blocks. This problem would not exist if the investigation could be conducted prospectively. The assessment of blast staining has the potential to be conducted independently for various subtypes of MDS and MPN. However, this was not undertaken to not to cause the data confusion. Due to the study's single-center design, the potential for a restricted number of instances exists. A potential enhancement to the study would involve conducting a prospective analysis with a larger sample size, which would likely yield more reliable and dependable findings.

CONCLUSION

The overall results of our study show that CD34 is still the most effective marker for the determination of blast count and grade in MDS, while CD117 may have a supportive role in this process and may be particularly useful in the identification of myeloid line progenitors as an ancillary marker. In MPN, CD117 expression is significantly higher than CD34 expression. It is undeniably beneficial, particularly in MPN cases with myeloid proliferation. Specifically, analyzing CD34 and CD117 simultaneously would be an excellent indicator for making the correct diagnosis.

β-catenin, which has subsequently started to be used in hematopoietic diseases, has a high performance in AML cases with the nuclear staining property but cannot show the same performance in MDS and MPN cases. The very limited number of studies conducted on this subject are unable to fully address the questions on the role of Wnt/β-catenin in diseases like MDS and MPN. Future clarification of the function of β-catenin in the diagnosis and treatment of hematological neoplasms will increase as the number of comprehensive studies on this topic increases.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval by the ??????????????Non-interventional Clinical Researches Ethics Committee (Date: 25.03.2021, Decision No: 2021.03.07).

Informed Consent: Written consent was obtained from the patient participating in this study.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Chronic lymphocytic leukemia

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ABSTRACT

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a mature B-cell neoplasm characterized by progressive accumulation of monoclonal B lymphocytes. CLL is considered being identical to SLL. Malignant cells seen in CLL and SLL have the same pathological and immunophenotypic features. The term CLL is used when the disease occurs mainly in the blood, while the term SLL is used when the involvement is mainly nodal.

Keywords: Chronic lymphocytic leukemia, small lymphocytic lymphoma, mature B-cell neoplasm

CHRONIC LYMPHOCYTIC LEUKEMIA

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a mature B-cell neoplasm characterized by progressive accumulation of monoclonal B lymphocytes.

CLL is considered being identical to SLL. Malignant cells seen in CLL and SLL have the same pathological and immunophenotypic features. The term CLL is used when the disease occurs mainly in the blood, while the term SLL is used when the involvement is mainly nodal.

EPIDEMIOLOGY

CLL constitutes approximately 25-35% of all leukemias. It is the most common leukemia among adults in Western countries.[1] It manifests as SLL, primarily in the lymph nodes, in less than 10% of patients. CLL/SLL is more common in men, with a male/female ratio of approximately 1.2:1 to 1.7:1 (1,2). Worldwide, there are approximately 191,000 cases of CLL/SLL per year, as well as 61,000 deaths from the disease .[3]

The median age of diagnosis of CLL/SLL is approximately 70 years. Although it is rare to occur before the age of 40, the incidence of the disease increases logarithmically after the age of 45. The incidence of CLL/SLL varies by race and geographic location. In the United States, there is a higher incidence among White Americans compared to African Americans or Asian Pacific Islanders.[4] It is extremely low in Asian countries such as China and Japan.[5-6]

ETIOLOGY

There are no clearly discernable occupational or environmental risk factors that predispose to CLL/SLL. The role of radiation and chemotherapy in the development of CLL has not been proven. Exposure to chemicals, such as solvents, benzene, dyes, and pesticides, is not a proven risk factor for CLL. Compared to other leukemias, familial occurrence is most prominent in CLL. Multiple CLL cases were observed at a higher frequency in a single family. Close to 10% of CLL patients have a relative with 1st or 2nd degree CLL. Although the majority of CLL cases precede monoclonal B-cell lymphocytosis (MBL), a small percentage of these people develop CLL. In addition, polymorphisms in CD5 (localized on chromosome 11q23), CD38 (localized in chromosome 4p15), the gene encoding TNF-alpha, and other genes mapping chromosome 13q21,33q22.2 are genetic factors that cause an increase in the incidence of CLL.

PATHOGENESIS

The pathogenesis of CLL/SLL is a complex process leading to the accumulation of monoclonal, mature, dysfunctional B lymphocytes in peripheral blood, bone marrow, lymph nodes, and spleen. (**Figure 1**).

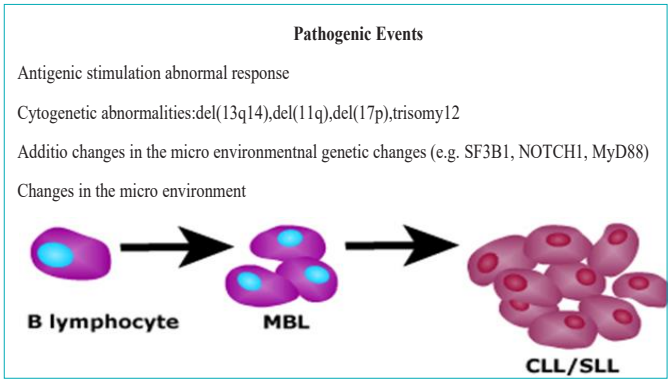

Figure 1. Pathogenesis of CLL-SLL

The pathogenesis of CLL/SLL is complex but appears to follow a two-stage pattern of progression. First, MBL develops as a result of genetic changes that are thought to be the

product of an abnormal response to antigenic stimulation. A secondary event, occurring randomly rather than cumulative damage, causes tumor cells to accumulate and progress to CLL/SLL. Signs and symptoms (hepatomegaly, splenomegaly, cytopenia, infection, lymphadenopathy) are associated with the accumulation of functionally dysfunctional lymphocytes in the bone marrow, lymph nodes, and other organs.

CLINICAL SYMPTOMS

Symptoms

Most patients are diagnosed with an absolute lymphocytosis during a routine blood count without any symptoms. Others are diagnosed with painless swelling in the lymph nodes, which usually grow and shrink spontaneously in the cervical region, but do not disappear completely, after examinations made during the visit to the doctor.

5-10% of patients may have B symptoms including one or more.[7] These are;

- Weight loss of 10% of body weight in the last six months
- Fever >38°C for ≥2 weeks without evidence of infection
- Night sweats without signs of infection
- Extreme tiredness (not able to work or do normal daily activities)

Findings

Lymphadenopathy

It is the most common abnormal finding detected on physical examination and is seen in approximately 50-90% of patients.[8-9] Patients may present with localized LAP as well as with diffuse LAP. The cervical, supraclavicular, and axillary lymph nodes are most commonly affected. Characteristically, the lymph nodes are painless, firm, round, and mobile with palpation. Sometimes, lymph nodes in the same anatomical region may merge (conglomerated LAP) to form large lymphoid masses.

Splenomegaly

Spleen enlargement is seen in 25-55% of cases.[8-9] It is the second most enlarged organ after lymph nodes. The enlarged spleen is usually painless and has a smooth surface with a sharp, hard edge on palpation.

Hepatomegaly

Liver size is detected in 15-25% of cases at the time of diagnosis.[8-9] It is usually 2-6 cm palpable from the right costal margin and is approximately 10-16 cm. On palpation, it is usually not sensitive and has a smooth surface.

Skin and other organ involvement

CLL/SLL lymph nodes can also be seen outside of the liver and spleen. The skin is probably the most frequently involved non-lymphoid organ for ease of examination. Skin lesions (leukemia cutis) are mostly seen in the face region and may present as macules, papules, plaques, nodules, ulcers, or vesicles.[10] Diagnosis can be made by skin biopsy. Skin involvement occurs in less than 5% of cases and may not significantly affect prognosis unless biopsy shows Richter's transformation.

Unlike other lymphomas, gastrointestinal mucosal involvement is rare. At the same time, although meningeal leukemia is very rare, its incidence varies between 0.2-2%.[11] Paraneoplastic renal involvement forms such as membranoproliferative glomerulonephritis (MPGN), minimal change disease, and amyloidosis can also be seen rarely .[12]

LABORATORY

Lymphocytosis

The most important laboratory abnormality in CLL is B-cell lymphocytosis in the peripheral blood and bone marrow. The absolute blood lymphocyte threshold for the diagnosis of CLL is >5000/microL (5x109/L) (12). A significant proportion of lone patients present with a lymphocyte count as high as 100,000/microL (100x109/L).

Sometimes, excessive lymphocytosis may cause complications due to increased hyperviscosity (e.g. transient ischemic attack, stroke).[13] However, there is no clear consensus on this threshold value. Particular attention should be paid to patients with other risk factors for cerebrovascular complications (e.g., hypertension, atherosclerotic disease) and hydration should be offered to these patients.

Cytopenias

Although not severe at the time of initial diagnosis, neutropenia, anemia and thrombocytopenia may be seen. In addition, autoimmune hemolytic anemia (AIHA), pure red cell aplasia (pure red cell aplasia, PRCA), autoimmune thrombocytopenia (ITP) and agranulocytosis may contribute to the development of cytopenia. The incidence of autoimmune hemolyticanemia is increased in patients with CLL. Direct Coombs (antiglobulin) test (DAT) positivity can be seen during the disease in approximately 35% of cases. Overt AIHA occurs in approximately 10% of cases and is usually later in the course of the disease.[14] Pure red cell aplasia occurs in approximately 0.5 percent of patients. Unlike AIHA, PRCA can occur early in CLL. Immune Thrombocytopenia (ITP) is seen in 2-3% of patients with CLL and may be the first finding that may be an indication for treatment.[14] Agranulocytosis can be seen very rarely at 0.5%.

Immunoglobulin abnormalities

Hypogammaglobulinemia is present in approximately 25% of patients at the time of diagnosis, and up to two-thirds of patients may develop it later in the course of the disease.[15] Usually, all three immunoglobulin classes (IgG, IgA, and IgM) are reduced, but in some patients only one or two is low. CLL patients are more susceptible to major bacterial infections when low immunoglobulin levels and neutropenia are present. Polyclonal increases in gamma globulins are seen in approximately 15% of patients.

Abnormal biochemical findings

Approximately 60% of patients with progressive or advanced CLL have increased levels of lactate dehydrogenase (LDH) and beta-2 microglobulin. Elevations in uric acid, hepatic enzymes (ALT or AST) and, rarely, calcium may also be observed.

PATHOLOGICAL CHARACTERISTICS

Peripheral smear

Lymphocytosis is seen in the peripheral blood smear of patients with CLL. Typically, most leukemic cells are mature-appearing lymphocytes with narrow basophilic cytoplasm, dense nuclei, no nucleoli (**Figure 2**). Occasionally,

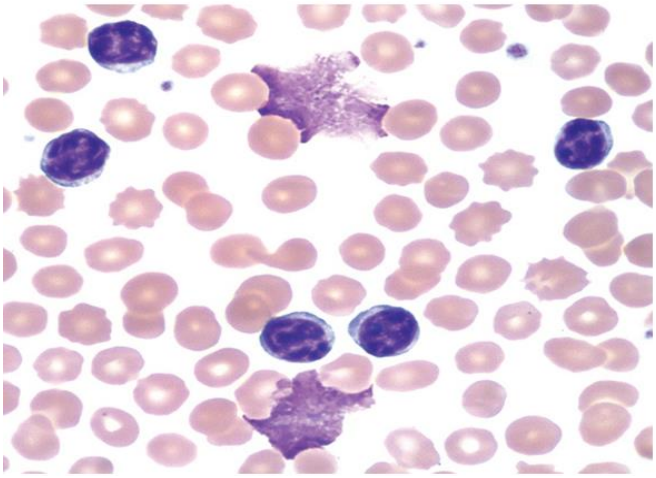

Figure 2. Chronic lymphocytic leukemia peripheral blood smear

some of the circulating cells are composed of medium-sized prolymphocytes with large or oval notched nuclei and prominent, single, central nucleoli, usually constituting a small fraction of the lymphocyte population. Also, the peripheral smear often contains "basket" cells (basket cells). These are lymphocytes that are mechanically disintegrated in the process of spreading onto the lamella, probably because CLL cells are more fragile than normal lymphocytes.[16]

Immunophenotype

Immunophenotypic analysis, usually performed by flow cytometry, is a key component in the diagnosis of CLL.[17] CLL cells carry CD5, except for B lymphocyte surface markers (CD19, CD20, CD23). Surface immunoglobulin (SmIg), FMC7, CD22 and CD79b are expressed lower than in either negative or normal B lymphocytes. Typically, only a single immunoglobulin light chain (kappa or lambda) is expressed. In addition, CLL cells are cyclin D1 and CD 10 (usually) negative. CD38 (>30%) is expressed in approximately 40% of cases.

BONE MARROW ASPIRATION AND BIOPSY

Bone marrow aspiration and biopsy are not required for the diagnosis of CLL. If bone marrow biopsy and aspiration are performed at initial diagnosis, they usually show normal to increased cellularity, with lymphocytes accounting for >30% of all nucleated cells. The bone marrow involvement pattern may be interstitial, nodular or diffuse (**Figure 3**).

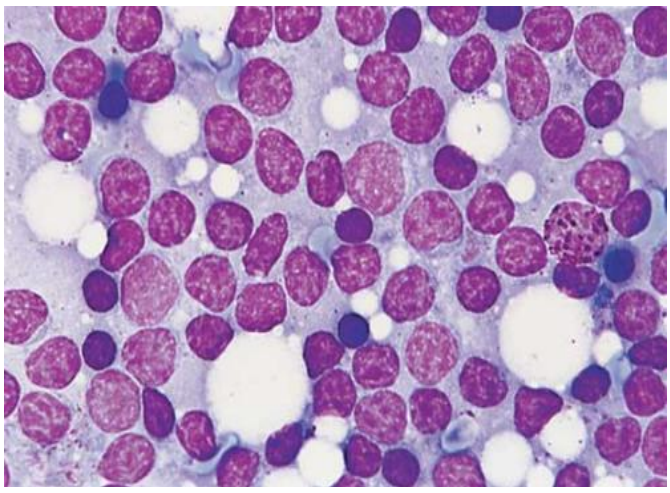

Figure 3. Diffuse involvement of the bone marrow by lymphocytes

Lymph node and spleen histology

The structure of involved lymph nodes is diffusely deleted, sometimes with scattered residual bare germinal centers. The lymph node infiltrate consists predominantly of small lymphocytes with dense chromatin, round nuclei and rarely a small nucleolus.[18] Larger lymphoid cells (prolymphocytes and paraimmunoblasts) with more prominent nucleoli and scattered chromatin are often present. These large lymphoid cells are usually collected in “pseudofollicles” (**Figure 4**). This condition is considered pathognomonic for CLL/SLL. In the spleen, it usually shows both white and red pulp infiltration, but white pulp involvement is usually more prominent.

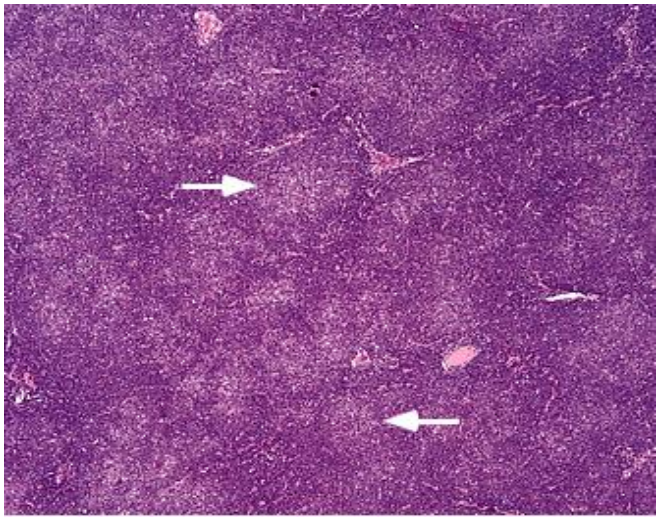

Figure 4. Lymph node biopsy in a patient with CLL

EVALUATION AND DIAGNOSIS

CLL is suspected in a patient presenting with an absolute lymphocytosis. Complete blood count, peripheral smear and flow cytometry should be requested for the evaluation of these patients. Bone marrow is usually not required but may be performed for the evaluation of patients with unexplained cytopenia. In contrast, the diagnosis of SLL usually involves painless swelling of the lymph nodes in the adult, usually in the cervical region, which can enlarge and shrink spontaneously, but does not disappear completely. Evaluation of such patients typically requires a tru-cut or excisional biopsy of a lymph node. For extranodal regions, biopsy of the relevant tissues and bone marrow biopsy and aspiration are performed in those with cytopenia. Regardless of the absolute lymphocyte count in the peripheral blood or the presence of lymphadenopathy, the demonstration of typical CLL cells in the bone marrow in the case of any cytopenia makes the diagnosis of CLL. In addition, SLL is diagnosed in patients with nodal, spleen or extramedullary involvement and no bone marrow involvement.

For the diagnosis of CLL, both of the following criteria must be met, according to the 2018 CLL National Cancer Institute guidelines.

- In peripheral blood; Absolute B lymphocyte count ≥5000/micro/L (5 x 109 / L), which is determined to be at least 3 months and morphologically mature small lymphocytes predominate in the peripheral smear,
- In peripheral blood flow cytometry; low SmIg levels, presence of B-cell associated antigens (CD19, CD20 and

CD23), demonstration of CD5 and immunoglobulin light chain (kappa or lambda) positivity.

DIFFERENTIAL DIAGNOSIS

The diagnosis of CLL is suspected when the peripheral blood in an adult shows an absolute lymphocytosis. However, lymphocytosis can occur in non-neoplastic conditions such as viral or other infections (e.g., infectious mononucleosis, pertussis, toxoplasmosis) as well as in neoplastic conditions other than CLL (e.g., leukemic phase of lymphomas, hairy cell leukemia). Therefore, for differential diagnosis; first, to distinguish between reactive causes of lymphocytosis and clonal (malignant) causes, and second, to distinguish CLL from other malignant lymphoproliferative diseases. A clear diagnosis of CLL can be made in a patient with peripheral blood lymphocytosis and a CLL immunophenotype on flow cytometry (Table 1).

The differential diagnosis of patients presenting with lymphadenopathy and without minimal or no lymphocytosis includes other lymphoid malignancies. In this case, the most reliable morphological distinction is the presence of proliferation centers in the relevant lymph nodes of SLL and the absence of other pathological findings. CLL/SLL may also develop into an aggressive histology (Richter transformation), which may sometimes be present at the time of diagnosis.

Causes of lymphocytosis

1. Infections: In case of infection (such as infectious mononucleosis, pertussis, toxoplasmosis), transient lymphocytosis can be seen in the peripheral blood of patients.

For a diagnosis of CLL to be made, lymphocytosis must last longer than 3 months. Unlike CLL, lymphocytosis due to infectious causes is not clonal, does not show the characteristic immunophenotype of CLL, and does not infiltrate the bone marrow.

In addition, atypical lymphocytes (T lymphocytes) are observed in viral infections.

2. Monoclonal B-cell lymphocytosis (MBL): Monoclonal B-cell lymphocytosis (MBL) is used for an absolute increase in the number of clonal B lymphocytes in the peripheral blood not exceeding 5000/microL (5 x 109 /L) in the absence of other signs of disease (e.g., lymphadenopathy, organomegaly, cytopenia).

3. Prolymphocytic leukemia (PLL): Prolymphocytes that differ from typical CLL cells are present in the peripheral smear or bone marrow. Compared to CLL cells, these are medium to large sized cells with vesicular nuclear chromatin, a prominent nucleoli, and moderate amounts of cytoplasm that appear somewhat immature. In PLL of B lymphocyte origin (B-PLL), prolymphocytes are of the B lineage, expressing glossy surface membrane immunoglobulin (SmIg) and generally not CD5 (Table 1).

4. Mantle cell lymphoma (MCL): MCL cells also express CD5 and CD20 together (Table 1). Also, in the vast majority of cases, MCL cells stain strongly for cyclin D1, express high levels of SmIg and CD20, have t(11;14) chromosomal aberration, and are negative for CD23. In contrast, CLL cells are negative for cyclin D1 and often CD23 positive.

5. lymphoplasmacytic lymphoma (LPL): Both LPL and SLL are lymphoproliferative disorders that usually progress slowly. LPL is synonymous with Waldenström macroglobulinemia, a hyperviscosity syndrome caused by high serum IgM levels. A minority of cases are CD5 positive and show increased monoclonal paraprotein. LPL can be distinguished from CLL by the absence of CD23 expression, the presence of strong staining for surface IgM and CD20, and the presence of cytoplasmic Ig (Table 1).

6. Hairy cell leukemia (HCL): HCL and CLL/SLL may be associated with high lymphocyte counts in peripheral blood, but leukocytosis in HCL is much less common and occurs in only about 10-20% of cases. HCL often presents with splenomegaly and cytopenia but almost never involves lymph nodes, whereas lymphadenopathy is almost always found in CLL/SLL. The diagnosis of HCL is often suspected based on the presence of circulating lymphocytes (hairy cells) with cytoplasmic projections (65). Classical forms of HCL are distinguished from CLL based on immunophenotypic findings such as "bright" staining for CD20 and surface immunoglobulin, positivity for CD25, CD11c, annexin A1 and CD103, and an inability to express CD5 in the majority of cases (Table 1).

7. Follicular lymphoma (FL): Follicular lymphoma (FL) patients, similar to CLL/SLL patients, may present with diffuse painless peripheral lymphadenopathy, often in a cascade over long periods of time. Both have small tumor cells. FL can be distinguished from CLL/SLL by immunophenotype. Unlike FL, tumor cells in CLL do not express CD10; in contrast, tumor cells in FL, unlike CLL, do not express CD5 (Table 1).

8. Splenic marginal zone lymphoma (SMZL): Both SMZL and CLL can present with splenomegaly and peripheral blood lymphocytosis. Also, both CLL and SMZL can express CD23, CD43, CD5 and IgD, although their expression is much more typical for CLL. Unlike CLL, SMZL may have bright SmIg and CD20 (Table 1). In difficult cases, pathological evaluation of the bone marrow, spleen and lymph nodes may be required to establish the diagnosis.

Table 1.Classical immunophenotype of low-grade lymphoproliferative diseases

	CD5	CD19	CD20	CD23	CD10	CD25	CD103	CD200	sIg
CLL	+	+	slightly glossy	+	-	+/-	-	+	slightly glossy
MCL	+/-	+	glossy	-/dim	-	+/-	-	-	glossy
FL	-	+	+	+/-	+	-	-	-	+
MZL	+/-	+	glossy	+/-	-	+/-	-	-	+/glossy
HCL	-	glossy	glossy	-	-	+	+	+	+
WM/LPL	+/-	+	+	+/-	+/-	+/-	-	+	+/variable
B-PLL	-	+	glossy	+/-	-	-	-	+/-	glossy

PROGNOSIS AND STAGING

Prognosis

The natural history of CLL/SLL is highly variable, with a survival time of approximately 2-20 years from initial diagnosis. Some patients experience rapid deterioration and die within 2-3 years from complications or from causes directly related to CLL/SLL. Other's progress clinically well for 5-10 years, followed by a terminal phase lasting 1-2 years. A small proportion (<30%) of patients have a good clinical course for 10-20 years and the ultimate cause of death may be unrelated to CLL/SLL. Spontaneous clinical regression has been reported very rarely in the absence of treatment.[19] In the asymptomatic stage, patients continue their daily lives, but in the advanced stage, the performance status is impaired due to repeated hospitalizations. The most common causes of death are due to severe systemic infection (especially pneumonia and septicemia), bleeding, cachexia and disease-related complications.

Staging

The Rai and Binet staging systems divide patients into 3 risk groups based on predicted overall survival (OS) (**Table 2-3**). Survival curves for Rai low risk (stage 0), intermediate risk (stage I or II), and high risk (stage III or IV) groups correspond to Binet grades A, B and C, respectively. Advanced stage is associated with shorter survival.

Treatment of CLL has improved markedly in recent years. Therefore, the prognosis has improved with the advent of new treatments.[20] The advanced stage causes deterioration of bone marrow function (anemia and thrombocytopenia), beginning in the blood and bone marrow (lymphocytosis), involving the lymph nodes (lymphadenopathy), spleen and liver (organomegaly). Over time, patients tend to progress from an early stage (low risk), to an intermediate stage, and eventually to an advanced stage (high risk). Imaging is not used for routine staging but should be performed in patients with enlarged abdomen or pelvic LAP related signs or symptoms. Cytopenias may be due to bone marrow involvement or may be due to autoimmune causes (such as AIHA, ITP). According to retrospective studies, patients with cytopenia due to autoimmune processes have a better prognosis than patients with cytopenia due to bone marrow failure .[21]

Rai Staging System

The Rai staging system uses physical examination and complete blood count to classify patients into 3 risk groups (**Table 2**). While the prognostic value of the Rai and Binet grading system continued, the estimated median AS increased significantly as treatments improved.

Binet Staging System

The Binet staging system uses physical examination and complete blood count to classify patients into 3 risk groups with different estimated AS (**Table 3**). To determine Binet stage, physical examination evaluates 5 potential sites of lymphoid involvement (cervical, axillary, and inguinal lymph nodes unilateral or bilateral, each area counted as one), spleen, and liver. Lymph node enlargement is defined as ≥1 cm.

In addition to Rai and Binet, genetic prognostic factors that can determine the prognosis, especially in the early stage, are also used in staging (**Table 4**). The patient group with 17p deletion has the worst prognosis with a mean survival of 2 years.[22] Although another bad prognostic factor is 11q deletion, this bad risk effect has been overcome with treatments such as FCR (fludarabine, cyclophosphamide, rituximab). The unmutated immunoglobulin heavy chain variable region gene (immunoglobulin variable heavy chain (IGHV)) also indicates the possibility of high-risk disease.

In addition to these two clinical and laboratory-based classifications, a new classification system, which includes genetic data, has started to come into use. In this new classification, which is called chronic lymphocytic leukemia-International Prognostic Index (CLL-IPI), 5 independent prognostic factors are used.[23] (**Table 5**). The risk groups evaluated in 4 different categories according to this system and their survival percentages are shown in **Table 6**.

Table 2. RAI staging System

Stage	Risk	Explanation	Average survival (months)
0	Low	Absolute lymphocytosis in peripheral blood or bone marrow (>5000/mm^3)	>150
I	Mild	Lymphocytosis and lymphadenopathy	101
II	Mild	Lymphocytosis and hepatomegaly and/or splenomegaly ± lymphadenopathy	71
III	High	Lymphocytosis and anemia (Hgb<11 g/dl) (and/orsplenomegaly, hepatomegaly, lymphadenopathy)	19
IV	High	Lymphocytosis and thrombocytopenia (platelet count <100,000/microL (and/or anemia, splenomegaly, hepatomegaly, lymphadenopathy)	19

Table 3. Binet staging system

Stage	Risk	Explanation	Average survival (months)
A	Low	<3 lymphatic region involvement	could not be reached
B	Mild	≥3 lymphatic region involvement (Hgb≥10gr/dl, platelet count≥100.000/mm^3)	84
C	High	Hgb<10gr/dl, platelet count<100,000/mm3 (number of areas with lymph node involvement is not important)	24

Table 4. Genetic risk factors	
Genetic marker	**Risk group**
Del 17p and/or p53 mutation	High risk
Del 11q	Medium-high risk
Del 13 q	Low risk
Trisomy 12	Medium risk
NOTCH1 mutation	Medium-high risk
SF3B1 mutation	Medium-high risk
BIRC3 mutation	High risk

Table 5. Chronic lymphocytic leukemia international prognostic index (CLL-IPI)	
Age	**≤65:0 point** **>65:1 point**
Clinical stage	Binet A/Rai 0: 0 point Binet B, C/Rai I-IV: 1 point
B2 microglobulin (mg/L)	≤3.5:0 point >3.5:1 point
IGHV mutation	Yes:0 point None: 2 point
Del 17p and/or p53 mutation	Yes:4 point None: 0 point
≥7 points: very high risk 4-6 points: high risk 2-3 points: medium risk ≤1 point: low risk	
CLL-IPI score	**5-year overall survival (%)**
Low risk	93.2
Medium risk	79.3
High risk	63.3
Very high risk	23.3

Table 6. Risk groups and survival rates according to the chronic lymphocytic leukemia international prognostic index (CLL-IPI)	
CLL-IPI score	**5-year overall survival (%)**
Low risk	93.2
Medium risk	79.3
High risk	63.3
Very high risk	23.3

TREATMENT IN CLL

Not all patients with CLL need treatment at the time of diagnosis. The main reasons for this are; Similar treatment outcomes of CLL between the normal population and certain patient subgroups, with the exception of allogeneic hematopoietic stem cell transplantation (AHSCT), the inability to cure CLL with current treatment options, no improvement in long-term survival with early treatment in patients treated early or late. Cannot be displayed .[24-25]

Pre-treatment Evaluation

History and physical examination,

Questioning systemic symptoms (fever, night sweats, weight loss, malaise)

Complete blood count and peripheral blood smear

Direct Coombs test and reticulocyte count.

Blood biochemistry: Glucose, blood urea nitrogen, creatinine, lactate dehydrogenase (LDH), uric acid, aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (ALP), albumin, bilirubin.

Serum protein electrophoresis, serum immunoglobulin levels (preferably),

Viral serological evaluation: All patients should be tested for human immunodeficiency virus (HIV), hepatitis B (HBV), and hepatitis C. In patients with chronically active HBV, antiviral therapy can be initiated before immunosuppressive therapy is administered to reduce the risk of reactivation of hepatitis B. Serology testing for cytomegalovirus (IgM and IgG) should be performed in patients treated with reactivation-related agents (e.g., idelalisib, alemtuzumab).

Performance evaluation,

Evaluation of electrocardiography and cardiac functions (left ventricular ejection fraction, rhythm disturbance),

Bone marrow biopsy and aspiration are not recommended at the time of diagnosis. Bone marrow biopsy can be considered in the diagnosis of immune-mediated or disease-related cytopenias.

t(11;14); t(11q;v); +12; del(11q); del(13q); It is recommended to examine the TP53, IGVH mutation status with del(17p),

and PCR/Sanger sequencing. Testing for del17p, TP53 mutation, and IGHV mutation status is critical to select appropriate therapy.

PA chest X-ray should be taken to evaluate hilar and mediastinal adenopathy. Computed tomography (CT) of the thorax, abdomen, and pelvis is not required for pre-treatment evaluation. It can be drawn to evaluate for compression complications, such as obstructive jaundice, obstruction of the inferior vena cava or ureters.

Pregnancy testing in women of childbearing age and men and women of childbearing potential should be counseled about the potential impact of treatment on their fertility and options for fertility preservation measures. Contraception is required during treatment.

Treatment indications

- Hemoglobin <10 g/dL or platelet count <100,000/microL(Rai stage III-IV; Binet stage C). Cytopenias unrelated to CLL should be excluded. In autoimmune anemia or thrombocytopenia, treatment is given for the autoimmune process. When there is no adequate response to the treatment, the treatment for CLL is started.
- Massive (ie, ≥6 cm below the left costal margin) or progressive or symptomatic splenomegaly.
- Massive (ie, ≥10 cm in longest diameter) or progressive or symptomatic lymphadenopathy.
- A greater than 50% increase in lymphocyte count in a two-month period or a lymphocyte doubling time (LDT) of less than 6 months.
- Symptomatic or extranodal involvement leading to loss of function (eg, skin, kidney, lung, spine).
- Patients with any or more of the following symptoms:
- Marked weakness and fatigue (ECOG PS ≥2)
- Excessive night sweats persisting for ≥1 month without evidence of infection
- Unintentional weight loss (more than 10% loss) within 6 months
- Fever above 38 °C lasting longer than ≥2 weeks without evidence of infection
- Absolute lymphocyte count alone is not an indication of treatment. Patients with mildly stable and asymptomatic cytopenias (neutrophil<1.000/mm3, Hb<10 gr/dL, platelet<100.000/mm3) can be followed closely without treatment.

Treatment approach in early stage asymptomatic (without active disease) CLL

For all patients with early-stage asymptomatic CLL (e.g., RAI grade <3, Binet grade A or B), regardless of risk factors (e.g. 17p deletion, unmutated IGHV), the standard of care is a wait-and-see approach rather than immediate treatment. Follow-up with hemogram and clinical examination every three months is recommended. If the disease is stable at the end of the 12-month follow-up, the follow-up period is extended to six months, and treatment is started for patients with an aggressive course and treatment indication.

Some patients with early-stage CLL need treatment within the first few years, while others remain asymptomatic for decades without treatment. Although variable, the median life expectancy in asymptomatic early stage CLL patients is more than 10 years.

Rarely, patients are present with a single enlarged lymph node (Localized SLL). In such a case, instead of recommending systemic chemotherapy or radiotherapy (RT) as in asymptomatic CLL, it is recommended to follow up until symptoms occur. Some experts recommend curative RT, such as early-stage Hodgkin's lymphoma (HL) and follicular lymphoma (FL). However, data on this condition are limited to small retrospective series .[26]

Treatment approach in advanced symptomatic (with active disease) CLL

There is no single agreed standard pretreatment regimen for all patients with symptomatic or advanced CLL. There are several initial treatment options. The goals of treatment are to improve symptoms, progression-free survival, and prolong overall survival. While overall survival rates are similar in different regimens, they differ in rates of complete remission, time to progression, and treatment-related toxicities. A choice among these treatments is made according to the characteristics of the patient, the characteristics of the disease and the goals of the treatment.

Treatment-free median survival in patients with advanced CLL (symptomatic or progressive) ranges from 18 months to 3 years. With current treatment regimens alone, the expected overall survival can vary from several years to decades, depending on disease characteristics, patient characteristics, and treatment choice.

Treatment selection

The choice of initial therapy for patients with symptomatic or advanced CLL is based on patient and tumor characteristics, patient preference, and goals of therapy. Currently, there is no single agreed standard treatment for CLL.

Treatment usually includes the following agents administered in combinations:

- Bruton tyrosine kinase (BTK) inhibitors (eg, acalabrutinib, zanubrutinib, ibrutinib)
- BCL2 inhibitor (venetoclax)
- Monoclonal antibodies (eg, rituximab, ofatumumab, obinutuzumab)
- Purine analogs (e.g., fludarabine, pentostatin)
- Alkylating agents (eg, chlorambucil, cyclophosphamide, bendamustine)
- Phosphoinositol-3-kinase enzyme (PI3K) (e.g., idelalisib, zandelisib, duvelisib)

Chemoimmunotherapy

There is no standard pretreatment regimen for patients with symptomatic CLL. A wide variety of combination chemotherapy regimens are used, often combining nucleoside analogues (e.g., fludarabine), alkylating agents (e.g., cyclophosphamide) and biological agents (e.g.,rituximab). The choice of treatment will depend on the person's age, risk of disease, and symptoms. Younger patients who receive FCR have longer progression-free survival than those who receive bendamustine and rituximab (BR). However, this is not the case in patients over 65 years of age, and BR is the preferred option for those who are not suitable for ibrutinib in this age group. The anti-CD20 monoclonal antibody obinutuzumab has been approved for a variety of indications in CLL.

Bruton tyrosine kinase inhibitors

Ibrutinib is an oral covalent inhibitor that binds to BTK and inhibits its kinase activity, leading to apoptosis of CLL cells. Approved for use in patients with CLL with/without prior treatment. Ibrutinib is used as primary therapy in patients with chromosome 17p13.1 deletions or TP53 mutations for whom immunochemotherapy is not

appropriate. Ibrutinib can also be given in combination with BR or obinutuzumab or rituximab.[27-28] Acalabrutinib is a more selective BTK inhibitor than ibrutinib and has demonstrated a good safety and efficacy profile in patients with relapsed/refractory CLL. Acalabrutinib monotherapy can be given alone or in combination with obinutuzumab to previously treated/untreated symptomatic CLL patients.[29]

Phosphoinositol-3-kinase enzyme (PI3K)

Idelalisib is a potent selective PI3K inhibitor that induces CLL cell death without affecting T and NK cells. It is approved for use in relapsed CLL in combination with rituximab and can be given to patients with poor prognostic factors.

BCL-2 inhibitors

Venetoclax is a highly selective inhibitor of BCL2, a protein essential for the survival of CLL cells. It has demonstrated an overall response rate of 70-80%, including patients with a 17p deletion or TP53 mutation for whom venetoclax can be given as monotherapy. Venetoclax can also be given in combination with ibrutinib, obinutuzumab or rituximab. Studies have shown that the first-line oral combination of venetoclax and ibrutinib provides complete remission after 12 cycles.[30]

OTHER TREATMENTS

Chimeric antigen receptor T cells (CAR-T Cell)

CAR-T cells are a genetically engineered T cell that targets an antigen (such as CD 19) found on the surface of CLL. It was developed and tested in clinical trials for patients with previously treated CLL.

Hematopoietic stem cell transplant

The role of hematopoietic stem cell transplantation in the treatment of CLL patients is decreasing after new effective agents for relapsed/refractory patients and high-risk patients. Although autologous stem cell transplantation has no place in CLL, allogeneic stem cell transplantation offers a curative approach in patients with CLL, but the timing and execution of stem cell transplantation is still debated given the availability of numerous new agents.

Splenectomy

It may be an option to cure refractory ITP and AIHA where medical therapy has failed.

Radiotherapy

It is an effective treatment method in local symptomatic LAP and isolated Richter transformation.

Leukapheresis

It can be applied in patients with CLL if there are signs and symptoms of hyperviscosity secondary to hyperleukocytosis.

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Review

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Cold autoimmune hemolytic anemia

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ABSTRACT

Cold agglutinins are antibodies that recognize antigens on erythrocytes at temperatures below normal body temperature. Antibodies are of IgM nature and bind to "I" or "i" antigens on red blood cells, causing agglutination in red blood cells. This situation results in anemia by creating extravascular hemolysis. If there is no underlying disease, it is called primary or idiopathic Cold Agglutinin Disease (CAD), and if there is, it is called Secondary Cold Agglutinin Syndrome (CAS). Primary CAD is an extremely rare disease, with an incidence and prevalence of 1 per million and 16 per million, respectively. It is seen twice more in women than in men, and the median age of diagnosis is 67 (between 30-92 years). The etiology of CAS includes infections, autoimmune and lymphoproliferative diseases. There are cold-related symptoms and symptoms of anemia in the clinic. In treatment, it is necessary to avoid cold in order to reduce cold-induced symptoms and hemolysis. Currently, the most effective treatment for reducing antibody production is Rituximab. It can be given alone or in combination with Bendamustine, Interferon alfa, Fludarabine and Prednisolone. Bortezomib is used when rituximab is ineffective or contraindicated. Plasmapheresis or intravenous immunoglobulin can be given when there is critical hemolysis or when the effectiveness of immunosuppressive therapy may start late. Treatment in CAS is the treatment of the underlying disease.

Keywords: Anemia, bortezomib, cold agglutinins, hemolytic anemia, rituximab

INTRODUCTION

CAD is an autoimmune hemolytic anemia that develops against "I" and "i" antigens on erythrocytes at temperatures below normal body temperature.[1,2] Cold agglutinins were first described by Landsteiner in 1903.[3] Clough and Richter described the relationship of cold agglutination with respiratory tract infections in 1918.[4] In 1943, Horstmann and Tatlock reported that they detected cold agglutinin in patients with primary atypical pneumonia.[5] The term CAD was first defined by Schubothe in 1966.[6]

Pathophysiology

It reacts with RBC antigens with polysaccharide epitopes on glycoproteins and glycolipids, such as cold agglutinins, ceramides or glycophorins. Antigens are "I" or "i" antigens on erythrocytes. About 99% of the population is "I" positive. Less than 1% are "i" positive and these individuals are also referred to as "I" negatives. Antibodies developed against these antigens are in IgM structure at a rate of 90%, and they may rarely be IgG, IgA or λ light chains, but in heat-associated autoimmune hemolytic anemia (AHA), antibodies are mostly IgG in nature.[7,8] IgM can extend the distance between RBCs and it is a 1 million-Da molecule that can overcome the natural repulsive forces between cells, thus allowing spontaneous in vitro agglutination. At normal body temperature, circulating IgM does not remain bound on the RBC surface. However, when blood slides into the peripheral circulation, it cools and IgM temporarily binds to the RBC surface. Once the IgM molecule binds, it activates the complement cascade that binds C3b to the cell surface. C1 activates and activates C4 and C2 with C1esterase, which is then cleaved into C3a and C3b by C3 convertase. C3b-coated RBCs are phagocytosed by macrophages in the reticuloendothelial system, mostly Kupffer cells in the liver, resulting in extravascular hemolysis.[9] Phagocytosis is the whole cell rather than part of the cell membrane, but may also involve a portion of the cell membrane in IgG-mediated autoimmune hemolytic anemia. While spherocytes are not observed in CAD, they can be observed in warm CAD. In RBCs that do not phagocytosis and remain in circulation, when the body temperature returns to normal, IgM decomposes, but C3b remains bound and undergoes cleavage of the C3b surface to C3d, which can be detected by direct antiglobulin (Coombs) testing, and a positive Coombs test for complement occurs from the first findings pointing to CAD.[10]

Terminology, Epidemiology

Cold-type autoimmune hemolytic anemia is called primary or idiopathic Cold Agglutinin Disease (CAD) if there is no underlying disease, and secondary cold agglutinin syndrome (CAS) if present.

1. Primary CAD is a very rare disease, its incidence and prevalence are 1 per million and 16 per million, respectively.

2. The prevalence of CAS is not known exactly, but has been estimated from various case series. In a study conducted in Denmark, cold agglutinins were detected in vitro in 407 (82%) of 496 subjects with M. pneumoniae infection, but it was not correlated with clinical symptoms or hemolysis, and most cold agglutinins were clinically silent, cold agglutinins developed in 5% of 217 individuals, but clinical hemolysis was detected in only 1.5%.[12] CAD constitutes approximately 15% of all autoimmune hemolytic anemias. It is seen twice more in women than in men, and the median age of diagnosis is 67 (between 30-92 years).

Causes of secondary cold agglutinin syndrome

The underlying diseases in CAS are examined under three main headings.

a. Infections: It most commonly occurs during EBV[13-16] and M. pneumonia17-20 infections. However, association with Legionella, Varicella,[21,22] HIV[23], Citrobacter[24], Influenza[25,26] and Rubella[27] has also been observed in case reports. Clinically significant hemolysis does not occur in the majority of patients in CAS that develop after infection. Cold agglutinins usually appear about two weeks after the onset of primary infection, subside as the infection begins to resolve, and resolve completely within two to three months.

b. Autoimmune diseases: Systemic sclerosis (scleroderma)[28] is observed in rheumatoid arthritis[29,30] and SLE.[31,32] In Sjögren's Syndrome, while CAD is quite rare, heat-associated autoimmune hemolytic anemia is usually observed.[33-35]

c. Malignancies: In a study involving 78 patients with cold agglutinins, 65% of the patients were associated with hematological malignancies: Lymphoma was found in 40%, WM in 17%, and Chronic lymphocytic leukemia (CLL) in 8%.[36] Hematologic malignancies were observed in 78% of 89 patients with agglutinins: Monoclonal gammopathy of Uncertain Significance (MGUS) in 47%, Non-Hodgkin B-cell lymphoma in 9%, indeterminate lymphoproliferative disease in 9%, and CLL in 4%.37 Apart from hematological malignancies, sarcoma[38] has also been described in patients with carcinoma[39] and melanoma[40], although it is thought that these cases may be coincidental rather than causal relationships. When cold agglutinin is a result of malignant proliferation, its plasma concentration can be used as a tumor marker. The antibody may disappear with appropriate treatment and may reappear with recurrence of the tumor.

CLINIC

In the clinic, patients are often asymptomatic, but in the study of Berentsen et al., more than 90% of patients reported cold-related symptoms.[2]

Cold-induced symptoms: It causes necrosis with acrocyanosis, livedo reticularis, Raynaud's phenomenon and, in severe cases, cutaneous ulceration. In addition, weakness, fatigue, exertion and resting dyspnea may occur due to anemia.

In the laboratory, anemia can be observed in the hemogram. However, if the hemolysis is mild or compensated by reticulocytosis, anemia may not occur. In large case series, mean hemoglobin levels are approximately 9 to 10 g/dL. In some cases, it has been observed to be within the normal range but as low as 4.5 g/dL.[41-43] MCV is normal, high or low, depending on the degree of reticulocytosis. However, if the sample is cooled during processing, it will falsely rise due to RBC agglutination. Leukocytes and platelets are typically normal.

Signs of hemolysis: Reticulocyte, LDH and indirect bilirubin increase, haptoglobulin decreases. However, hemoglobinuria, which is a sign of intravascular hemolysis, is not observed. The complement-specific C3d is positive in the Coombs test, but if the cold agglutinin is accompanied by an IgG antibody, the Coombs test may also be weakly positive for IgG. This has been reported in approximately 25% of patients. In the study of Chandesris et al., it was reported that anti-C3d antibodies (74%), anti-C3d antibodies + IgG (22%) and only IgG (3%) were positive.[44] On the peripheral smear, agglutination of erythrocytes is evident, sometimes these agglutinations can also be seen in the blood tube. Typical cold agglutinin titers in CAD are quite high. The accepted threshold is > 1:64. In addition to the titer of cold agglutinin, its thermal amplitude is also important. It reflects the temperature range at which the antibody will bind to the RBC antigen. Most clinically important cold agglutinins have a thermal amplitude exceeding 28 to 30°C.[45] Thermal amplitude is more important than the titer for clinical manifestations, but is extremely difficult to measure in the laboratory.

DIAGNOSIS

Evidence of hemolysis (high reticulocyte, LDH, indirect bilirubin and low haptoglobin), positive direct Coombs test for C3d, and a cold agglutinin titer of ≥64 at 4°C are crutial. After diagnosis, infectious, autoimmune diseases and malignancies should be investigated.

Differential diagnosis

It is necessary to keep in mind the causes of cold-related symptoms and other hemolytic anemia. Cold-related symptoms are also observed in Reynaud's phenomenon and cryoglobulinemia, but the peripheral smear does not show agglutination in RBCs and there are no signs of autoimmune hemolytic anemia. WAHA is the most frequently confused in the differential diagnosis, the most common immune hemolytic anemia, but cold-induced symptoms are absent. Also, the Coombs test is positive for IgG instead of complement, and the smear shows spherocytes but no agglutination. Paroxysmal cold hemoglobinuria is a cold-induced autoimmune hemolytic anemia associated with IgG antibody against anti-P in RBCs; The Donath-Landsteiner test is positive and there is usually a recent viral infection in the history. The Coombs test may be positive for bound complement, but the cold agglutinin titer is minimally elevated (<1:160). Acute or delayed hemolytic transfusion reactions may also be confused, but the peripheral smear shows no agglutination of RBCs and no cold-induced symptoms.

TREATMENT

Avoiding cold, improving anemia, reducing antibody production and treating the underlying disease in CAS are the main principles. Avoidance of cold is very important to reduce cold-induced symptoms and hemolysis. It is necessary

to stay away from cold environments, food and drinks. Intravenous solutions and blood products should be brought to the appropriate temperature and used before infusion. However, in order to avoid thermal hemolysis, it should not exceed 40°C. When hospitalized patients have fever, they should be treated with antipyretics and, if necessary, antibiotics instead of cooling blankets (cold application).

Patients with mild, compensated hemolysis and stable anemia do not require transfusion for the treatment of anemia, while those with severe or symptomatic anemia may require transfusion. Blood products should be brought to the appropriate temperature, and the patient, especially the extremity to which the transfusion is applied, should be kept warm. Plasmapheresis or intravenous immunoglobulin (IVIG) treatment can be applied when there is critical hemolysis or the effectiveness of immunosuppressive therapy may start late. Since cold agglutinins are intravascular antibodies in the IgM structure, plasmapheresis is quite effective. Large-scale studies are not available to evaluate the efficacy of plasmapheresis in CAD, but case reports have shown it to reduce hemolysis and improve severe symptoms.46 The American Apheresis Society has defined therapeutic apheresis as category 2 for severe CAD. While plasmapheresis can remove existing IgM, it cannot affect IgM production, so it should only be viewed as a temporary measure until other treatments are effective. In preoperative patients, it should not be done more than 1-2 days before the procedure, this time is sufficient for the antibodies not to accumulate again, and the half-life for IgM to re-accumulate is about five days. Cryofiltration apheresis is a form of plasmapheresis that allows cold agglutinins to be removed without affecting other plasma proteins, and therefore does not require a replacement fluid such as donor plasma or albumin.47 IVIG clears cold agglutinin antibodies, but there is less experience in its use as its efficacy has not been clearly demonstrated, but some case reports has been reported.[7,48]

Treatment to reduce antibody production consists of regimens containing rituximab or bortezomib that target lymphoproliferative disorder in the bone marrow. Cold agglutinins are monoclonal in CAD while polyclonal in CAS. Treatment in CAS is the treatment of the underlying disease. Especially in CAS secondary to infection, cold agglutinins resolve spontaneously 2-4 weeks after infection treatment. Unlike warm AHA, glucocorticoids and splenectomy are not effective treatments for CAD. Glucocorticoids can reduce phagocytosis, but they cannot inhibit antibody production. However, sometimes IgG may accompany, in these cases, glucocorticoids may be added to the treatment. Splenectomy is also ineffective because in CAD the main site of RBC phagocytosis is the liver, not the spleen.

Rituximab is currently the most effective treatment, although slightly less effective than warm autoimmune hemolytic anemia (WAHA) in reducing antibody production.[49] Randomized studies have not been conducted to evaluate its efficacy, but case series have reported response rates of more than 50% and most responses are partial. In a 2006 study, rituximab was used in 52 of 86 patients with CAD; response rates were observed as 67%, complete response to combined therapy was better than single agent rituximab, and rituximab-based therapy was observed to be more effective than other immunosuppressive or cytotoxic agents.[2] In another study conducted in 2013, 89 patients with CAD had response rates of 83% for rituximab-based therapy (single agent) and 79% for combination therapy. These rates were higher than most other immunosuppressive or cytotoxic agents.[37] Similar response rates have been reported with rituximab alone or in combination therapy in small series.[49-51] The recommended dose for single-agent Rituximab is 375 mg/m^2 one day a week for four weeks.

In combination therapy with rituximab, bendamustine, fludarabine, corticosteroids and interferon alfa (INF-∝) are tried. In a prospective study of 45 patients treated with the Rituximab and Bendamustine regimen, 32 patients (71%) had a response and 18 (40%) had a complete response. Grade 3 neutropenia was observed in 15 patients (33%) and infections in 5 (11%).[51] And in a prospective study of 29 fludarabine-treated patients (10 with no prior single-agent rituximab response), the response was 76%, and the complete response was 21%. However, neutropenia was observed in 14%.[52,53] In the combination of rituximab and INF-∝, INF-∝, 5 million U were administered 3 days a week. In a series of 27 patients, the response rate was 54% and no serious side effects were observed.[54]

Bortezomib is a proteasome inhibitor used in B-cell lymphoid malignancies and its use is recommended in cases where rituximab is ineffective or contraindicated. In a study of 21 patients with CAD in whom at least one treatment was ineffective for the efficacy of bortezomib[55], six (32%) of 19 evaluable patients had a response (defined as improved transfusion dependence or a 2 g/dL increase in hemoglobin level), 3 of 6 patients had a complete response. While there was a response, partial response was observed in the other 3. Two different case reports were also observed in agreement with this study.[56,57]

Apart from rituximab and bortezomib regimens, anti-complementary therapy in CAD is being investigated. In CAD, the classical complement pathway is activated and causes extravascular hemolysis. Therefore, C1q is the target in anti-complementary therapy because it has enzymatic activity and is upstream of the classical pathway component. TNT009 is a humanized monoclonal antibody targeting C1q (based on a previous mouse monoclonal antibody TNT003) evaluated for reduction of C3b-mediated extravascular hemolysis.[58-60] A peptide inhibitor of C1q, reduction of hemolysis in CAD.[61] Anti-complementary therapies, such as eculizumab targeting distal complement component C5, are not expected to reduce extravascular hemolysis associated with early complements of the classical complement pathway, although some reports have also been shown to reduce hemolysis in selected patients.[62] However, these complement-directed therapies is not expected to improve cold-related symptoms associated with RBC agglutination because agglutination is mediated by IgM molecules.

Sutimlimab is a monoclonal antibody that targets C1 and was approved for the treatment of CAD in late 2022.[63] Pegcetacoplan is a pegylated cyclic peptide C3 inhibitor.[64] In both of these new treatments, vaccination is recommended for encapsulated bacteria before starting the treatment.

Summary

- CAD is a rare autoimmune hemolytic anemia.
- When there is an underlying cause, it is called CAS.
- When cold agglutinins are detected, especially infections, autoimmune and lymphoproliferative diseases should be investigated.
- Rituximab is still the most effective agent in treatment.

• In cases where rituximab is ineffective, combination treatments and Bortezomib are tried.

• CAD should be kept in mind in patients with hemolytic anemia, cold-related symptoms, and agglutinations of erythrocytes in peripheral smear.

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Case Report

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A rare case of acute basophilic leukemia

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ABSTRACT

Acute basophilic leukemia is an extremely rare form of acute myeloid leukemia and diagnosis is based on morphological, cytochemical and ultrastructural analyses of basophils and blastic cells. It is usually characterized by a very rapid clinical course, symptoms of hyperhistaminemia and resistance to therapy. A 73 year-old male presented with itching, thrombocytopenia and leukocytosis. There was no hepatosplenomegaly. In the flow cytometry evaluation of peripheral blood, CD123 positive myeloid blasts were detected at the rate of 60%. Also, CD13 and CD33 were positive, while CD34, CD117, HLA DR and myeloperoxidase (MPO) were negative. These findings were considered as consistent with ABL. Following, the diagnosis of ABL was confirmed by bone marrow evaluation including cytomorphological and immunohistochemical studies. On the other hand, cytogenetical analyses of bone marrow showed the presence of the Philadelphia chromosome. The patient received one cycle of a chemotherapy regimen including cytarabine and idarubicin with imatinib support, but he died on the 10th hospital day. Considering that ABL behaves in a very malignant fashion, and a more aggressive treatment approach is necessary for patients with this specific subtype of AML.

Keywords: Acute basophilic leukemia, CD123 antigen, flow cytometry

INTRODUCTION

Acute basophilic leukaemia (ABL) is an uncommon form of acute myeloid leukemia (AML) accounting for 4-5% of all cases.[1] Consistent diagnostic criteria for ABL still remain the topic of discussion. For diagnosis, it is necessary to exclude other hematological malignancies associated with basophilia such as chronic myeloid leukemia (CML), acute promyelocytic leukemia (APL) with basophilic differentiation, and AML/myelodysplastic syndrome (MDS). On the other hand, most cases of ABL have been reported to be a consequence of the evolution from other leukemias. In most cases, the prognosis is poor. Here, we report a rare case of ABL with a very agressive clinical course.

CASE REPORT

A 73-year-old male presented to hematology department with itching lasting for 3 months. He had no past any medical history, family history and history of tobacco, alcohol, illegal drug use, and travel. Also his past medical history was negative for atopic diseases. His vital signs were blood pressure 110/70 mmHg, pulse 88/min, respiratory rate 23/min, and body temperature 36.7 °C on admission. There was no hepatosplenomegaly or lymphadenopathy. Laboratory studies revealed the following: hemoglobin of 13.8 g/dL, white blood cells of 63x109/L, and platelets of 84x109/L. Serum biochemistry and coagulation were unremarkable.

There were large population of basophils on the peripheral blood smear. Viral, bacteriological and autoimmune evaluations were normal. Also, cytomegalovirus, rubella virus, herpes simplex virus, varicella zoster virus, Epstein–Barr virus, hepatitis markers and brucellosis tests were found as negative. In addition, analysis to detect a parasitic infection or an atopic cause were negative.

Spleen size was normal in the abdominal ultrasonography. Following, flow cytometry (FC) evaluation was performed by using FACS Calibur flow cytometer (Becton–Dickinson, Erembodegem, Belgium). In the FC evaluation of peripheral blood, CD123 positive myeloid blast cell group was detected at the rate of 60% of the total localized at CD 45 dim (Figure-1).

CD4: CD8 ratio was 65:33. Also, CD13 and CD33 were positive, while CD34, CD117, HLA DR and myeloperoxidase (MPO) were negative. These results were considered as consistent with ABL. Following, bone marrow biopsy was performed, and the aspirate revealed high cellular marrow

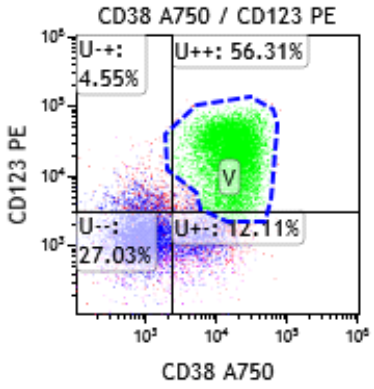

Figure 1. Shown is the 60% of CD-123 positivity in flow cytometry.

and most of the cells were CD13 and CD33 positive (Figure-2).

MPO was positive in only residual normal granulocytes

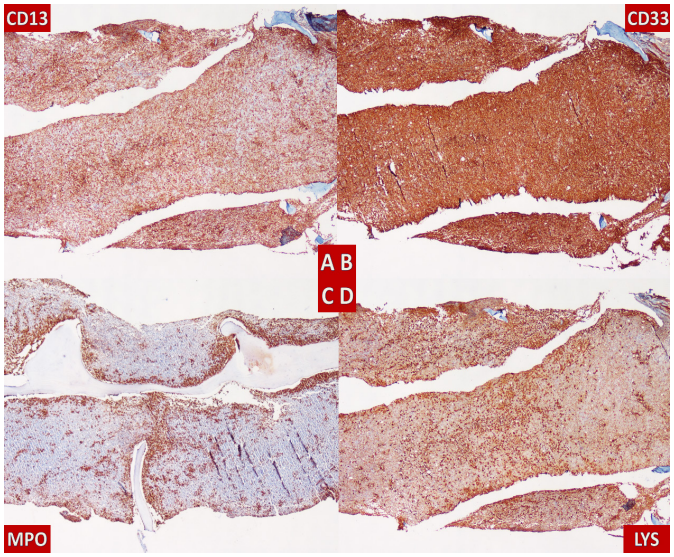

Figure 2. Immunohistochemical results. Most of the bone marrow cells are CD13 (A) and CD33 (B) positive. MPO is positive(C) in only residual normal granulocytes but lysosyme is positive (D) in both in normal granulocytes and some neoplastic cells (all sections are x40).

but lysosyme was positive in both in normal granulocytes and some neoplastic cells. Also, the blasts were negative for CD34, CD117, CD10, TdT and glycoforin. In the histopathologic sections of biopsy, mature erithroid and granulocytic cell ratio was diminished and some cells with abundant cytoplasm, indented ovoid nuclei were raised. There was a mild reticulin fibrosis and there was no siderophage in bone marrow. With these findings, bone marrow biopsy was reported as ABL.

Cytogenetic studies performed on bone marrow aspirate using standart culture methods and banding technique revealed t(9;22) and BCR/ABL1 fusion gene was detected by FISH analysis.

The patient was treated with a standart 7+3 regimen including cytarabine and idarubicin with imatinib support. Also, he received histamine receptor (HR) antagonists (HR1 and HR2 blocker) for itching. However, he died on the 10th hospital day due to aggressive progression of ABL.

DISCUSSION

Acute Basophilic Leukemia (ABL) has been known for a century. However, consistent diagnostic criteria are lacking. Therefore, most cases previously reported as ABL represented a variant of CML or developed secondary to MDS. Following, de novo ABL cases have been reported in recent years. Later, WHO classifications, ABL has been integrated as a ABL is an aggressive and extremely rare type of leukemia and is now classified as a distinct entity in the WHO classification. entity and defined as an AML in which primary differentiation is towards the basophil lineage.[2] However, no generally accepted criteria for ABL have been presented to date. To address this issue, in 2017, Valent et al. proposed a diagnostic algorithm of basophilic leukemias.[3] According to their recommend, for the diagnosis of basophilic leukemia, the percentage of basophils must be ≥ 40 of total leukocytes. In our case, this ratio was 60%. Also, they suggested the CD123 positivity for the diagnosis. In our case, CD123 (+) blastic cells group ratio was 60% in the FC analysis. In addition, they suggested to considered other causes of basophilia such as chronic infections, autoimmune processes and atopic disorders. In our case, investigations to detect these possible causes were negative. Also, they proposed the bone marrow examination for differential diagnosis of ABL. In our case, bone marrow biopsy resulted as ABL.

On the other hand, the Philadelphia chromosome had been described in some cases of MDS.[4,5] In our case, there were no any findings of cytomorphological evaluations compatible with MDS.

Actually, CML is the first diagnosis proposed in our patient due to positive Philadelphia chromosome, however, there was no splenomegaly, and the patient did not have positive history to CML, on the other hand, the rapidly progression, positive myeloid markers (CD13 and CD33), and positive CD123, make the diagnosis of ABL. Already, the diagnosis was confirmed by detailed bone marrow evaluation including cytomorphological and immunohistochemical studies. Even if our patient was diagnosed with CML, he still had ABL diagnosis according to current criterias. However, in this case it is called secondary ABL. In any case, whether it was primary or secondary, he had a diagnosis of ABL.

According to the literature, the survival time in ABL patients ranges from 2 to 16 months.[6] Therefore, these patients are often regarded as candidates for allogeneic stem cell transplantation (ASCT). However, not all cases are eligible for ASCT. Also, there are no prospective trials comparing treatment options for ABL. Several authors have reported results of various induction therapies for ABL. These have included treatment used for AML and acute lymphoblastic leukemia. However, the results have been disappointing.[7] Similarly, our case received one cycle of induction chemotherapy including cytarabine and idarubicin, but he died on the 10th hospital day due to aggressive progression of ABL.

Due to these results found in our case, ABL behaves in a very malignant fashion, and more aggressive treatment approach is necessary for this patients.

Learning points

ABL is an aggressive and extremely rare type of leukemia and is now classified as a distinct entity in the WHO classification.

It is important to differentiate ABL from CML, as in our case, the percentage of basophils above 40, CD 123 positivity and the absence of hepatosplenomegaly are important in the diagnosis of ABL.

There is still no clear algorithm in ABL treatment approach, so further studies are needed in this area.

ETHICAL DECLARATIONS

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Case Report

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Association of amyloidosis and lung cancer; a case of small cell lung cancer masked by amyloidosis with 18-fdg uptake

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ABSTRACT

Amyloidosis is a rare and fatal disease that occurs with the accumulation of amyloid protein in the organs. The most common presentations are nephrotic syndrome, cardiomyopathy, and unexplained hepatomegaly. We present a case of small cell lung cancer as a result of mediastinoscopic biopsy in a patient who presented with a lung mass and 18-FDG uptake on PET/CT, and amyloidosis was found in the bronchoscopic biopsy result.

Keywords: Lung cancer, 18-fdg uptake, amyloidosis

INTRODUCTION

Amyloidosis is a rare and fatal disease that occurs with the accumulation of amyloid protein in the organs. The most common presentations are nephrotic syndrome, cardiomyopathy, and unexplained hepatomegaly. Pulmonary involvement can be localized or systemic, primary or secondary, hereditary or acquired.[1] The incidence of pulmonary amyloidosis is uncertain because many cases are diagnosed incidentally during open lung biopsy or autopsy.

Lung cancer is the most common and deadliest type of cancer in the world.[2] Small cell lung cancer constitutes 10-15% of lung cancers. Generally, the disease has metastasized to other organs in most of the patients at the time of diagnosis.[3] Amyloidosis due to lung cancer is rare among the causes of secondary amyloidosis.

18-FDG PET/CT is accepted as an important diagnostic tool that is widely used in the detection of potentially malignant lesions.[4]

We present a case of small cell lung cancer as a result of mediastinoscopic biopsy in a patient who presented with a lung mass and 18-FDG uptake on PET/CT, and amyloidosis was found in the bronchoscopic biopsy result.

CASE

A 71-year-old male patient had complaints of chest pain, cough, weight loss, night sweats and shortness of breath, and was undergoing dialysis 4 times a week due to chronic kidney failure on the basis of amyloidosis. In his physical examination, a decrease in breath sounds was detected in the lower region of the left lung on auscultation. A cardiology consultation was requested due to the patient's chest pain development. No signs of cardiac amyloidosis were found in the ECG and echocardiography of the patient. The patient's laboratory values were WBC:16,500 (mm^3) (4.5-11,500), CRP (mg/L): 73 (0-5), Urea (mg/dl): 97 (18-55) Creatinine (mg/dl): 4.4 (0.72-1.25). In the thorax CT of the patient, a 9*7 cm mass in the upper lobe of the left lung, pulmonary artery invasion, mediastinal lymphadenopathies, and a pleural effusion reaching 3 cm thickness in the left hemithorax were detected (Picture 1). With these findings, primarily malignant diseases were considered. PET-CT was taken to reveal the extent of the disease. The PET-CT report resulted as follows. An increased FDG uptake was noted in the 87x108 mm mass lesion in the paramediastinal area (SUVmax 5.1) in the hilar region of the left lung, and the 35x41 mm nodal lesion (SUVmax 4.1) in the subcarinal region. Increased FDG uptake in the bone tissue, bilateral mandible, right hyoid bone, sternum, clavicles, costae, columna vertebralis, humerus, pelvic bones, and femurs, which erodes the bone tissue in diffuse foci and in places in the form of soft tissue infiltrations (SUVmax 7.3) has been noted. These findings included primary focus,

metastasis, multiple myeloma, and amyloidosis among the preliminary diagnoses (**Pictures 2-4**). The patient underwent a brain MRI for brain metastases. In the patient's brain MRI, there was a 10 mm-sized T1 isointense, T2 slightly hyperintense lesion showing diffusion restriction was observed in the right temporal cerebral cortical area. Also, irregularly circumscribed solid masses measuring 4.5x4 cm on the right and 5x4.5 cm on the left were observed in the bilateral mandible. These lesions were found to be compatible with metastasis and amyloid involvement.

We performed a bronchoscopy on the patient as the primary diagnostic procedure. On bronchoscopy, a mass was seen at the entrance of the left upper lobe and upper division of the lung. From here, biopsies and lavage were taken. Microbiological tests were studied in bronchoscopic lavage. No pathogen was detected. According to the pathology report, there is evidence of lymphoplasmacytic inflammatory cell infiltration and vascular proliferation in the stroma, along with alveolar macrophages and bronchial epithelial cells containing pigment-loaded large cytoplasm. The report states that there are no indications of atypia or malignancy, and the results are in keeping with the presence of amyloidosis and chronic nonspecific inflammatory findings.

In the case whose pathology result was amyloidosis, since the clinical and radiological suspicion of cancer continued, lymph node excision was performed from mediastinal lymphadenopathies in an external center. The pathology result was small cell lung cancer.

The patient received chemoradiotherapy treatment but passed away three months after diagnosis.

DISCUSSION

The incidence of pulmonary amyloidosis is uncertain because many cases are diagnosed incidentally during open lung biopsy or autopsy.

Little is known about the relationship between amyloidosis and the neoplastic state. Intratumoral amyloid deposition may contribute to the pathogenesis of the neoplastic condition.[5] Carcinoma-associated antigens can induce aggregation of amyloidogenic immunoglobulin light chain and nodular lesions.[6] The incidence of systemic AA amyloidosis in cancer patients is 0.1% to 0.4%.[7] In the series of 4033 lung cancer cases, only 1 amyloidosis case was detected).[8] In view of the small number of reported cases, the correlation between the pathological type of lung cancer and amyloidosis is unknown. High serum concentrations of SAA have been found in associated with the dissemination of lung cancer.[5] Only two cases have been reported of patients with pulmonary squamous cell carcinoma who developed systemic AA amyloid deposition after receiving treatment with chemoradiation and an immune checkpoint inhibitor.[1] Our case was diagnosed with systemic AA amyloidosis and small cell lung cancer developed during follow-up. Small cell lung cancer is the third most common lung cancer, and most patients metastasize to other organs at the time of diagnosis .[3] In our case, there were several metastases present.

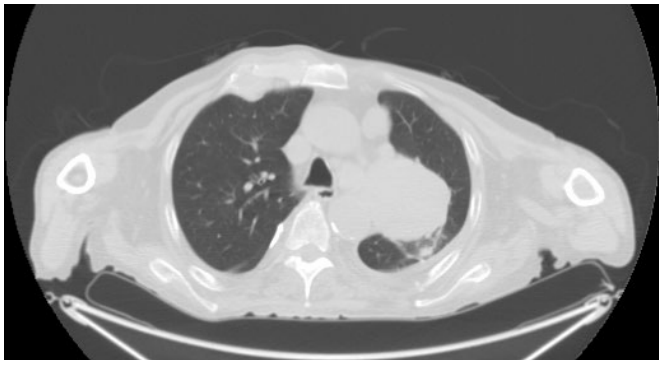
Positron emission tomography (PET) is used for differentiating benign and malignant lesions, as well as staging and monitoring response to chemotherapy. However, PET may show increased involvement in infectious and inflammatory diseases like sarcoidosis, tuberculosis, fungal infection, and cerebral abscess, leading to false positive results in oncological PET/CT studies.[9] Inflammatory diseases can cause false positive PET/CT results. FDG uptake occurs in areas with inflammation due to increased glucose uptake by macrophages and inflammatory cells. The threshold value for SUVmax is 2.5 for evaluating 18-FDG uptake. A higher standardized maximum uptake value increases the possibility of malignancy in lung and mediastinal lesions.[10,11] High SUVmax uptake in our case of amylodosis caused the suspicion of malignancy to continue.

A few case reports mention patients with nodular pulmonary amyloidosis who underwent 18F-FDG PET/CT, most of whom showed increased FDG uptake. In a study evaluating the role of 18F-FDG PET/CT in a group of patients with both systemic and localized amyloidosis, 18F-FDG uptake was observed in all patients with localized amyloidosis, but not in all patients with systemic amyloidosis. It has been shown that PET/CT can be useful in disease management and decision-making before biopsy is performed in patients with pre-diagnosed amyloidosis with pulmonary nodules.[12]

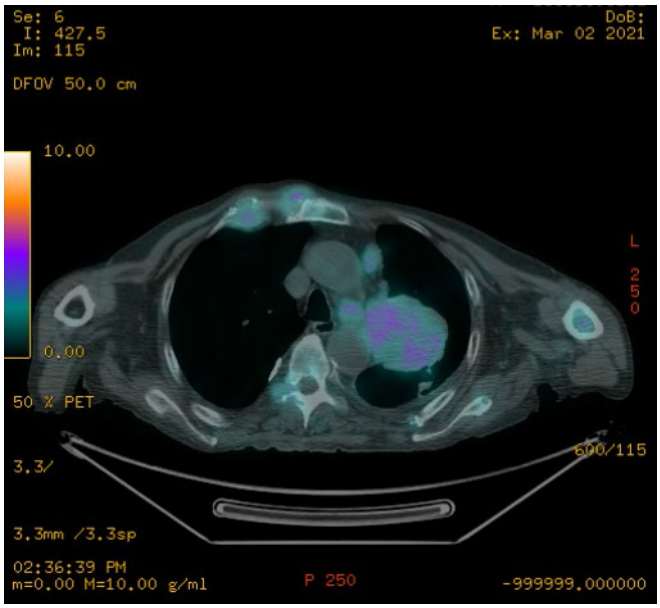
CONCLUSION

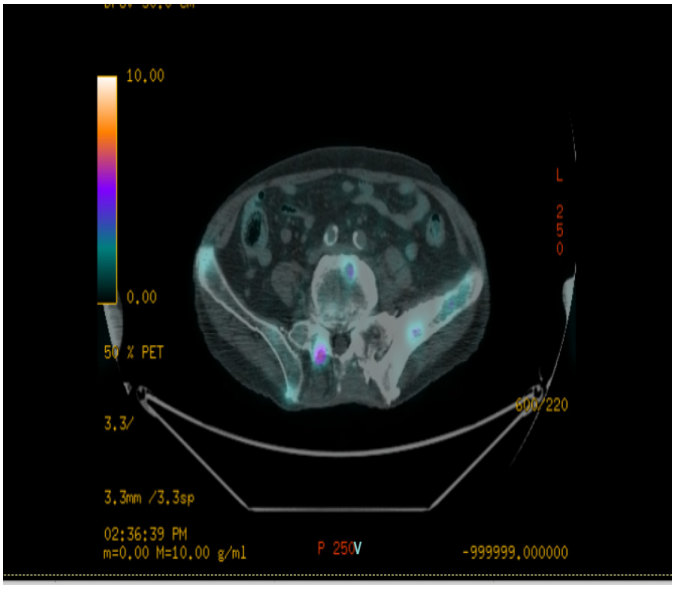
Pulmonary nodules; It is seen in a variety of lung diseases, including tumor, tuberculosis, and infection, but amyloidosis is rarely considered a candidate for the differential diagnosis of such lesions. Coexistence of lung cancer and amyloidosis is rare and should be kept in mind in the differential diagnosis.

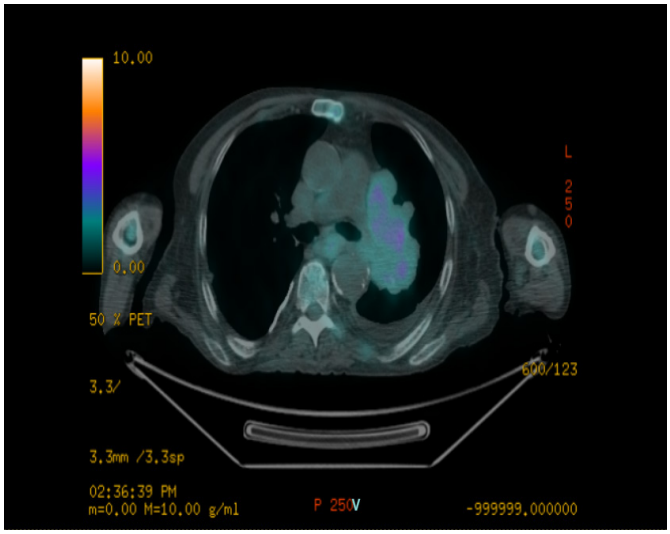
1. Thorax CT Cross-section



2-4.PET/CT Cross-sections







ETHICAL DECLARATIONS

Referee Evaluation Process: Externally peer-reviewed.

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