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THE DIFFERENCE IN PROTEIN PROFILES OF B CELLS AND T CELLS
BETWEEN APLASTIC ANEMIA AND MYELODYSPLASTIC SYNDROME
PATIENTS

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ABSTRACT

Aplastic anemia (AA) and hypocellular myelodysplastic syndrome (MDS) are two common acquired bone marrow failure diseases. AA is mostly an acquired bone marrow disease caused by cellular and humoral mediated immune attack of hematopoietic stem cells (HSC) due to dysregulation of immune system, which leads to hematopoietic stem cell and progenitor cell immaturely apoptosis and bone marrow failure. MDS is a group of acquired heterogeneously clonal HSC disorders with ineffective hematopoiesis. Approximately 10% to 20% of MDS manifests a reduced bone marrow cellularity, which comprises hypocellular MDS. There is increased experimental and clinical data indicating that an immune-mediated insult to hematopoietic HSCs and changes in the hematopoiesis-supporting microenvironment contribute to the pathogenesis of hypocellular MDS. Because of the similarity of their bone marrow manifestation, hypocellular MDS and AA are often hard to distinguish. Mounting evidence indicates that abnormal activation of cytotoxic T cells plays a crucial role in the pathophysiology of these diseases. However the difference in immune dysregulation in these two diseases, especially the role of B lymphocyte population, has not been thoroughly studied. Our study aims to find unique B lymphocyte surface marker expression patterns of hypocellular MDS and AA to help us understanding the pathogeneses of these two diseases.

This study retrospectively analyzed flow cytometry lymphocytic antigen expression profiles from patients diagnosed as AA and hypocellular MDS as per standard criteria. A total of 31 AA and 26 hypocellular MDS patient cases were recruited. The bone marrow aspirate/biopsy data, bone marrow aspiration flow cytometry reports, and

Complete Blood Counts (CBC)s from individual patients were analyzed. Using side scatter (SSC) vs. CD45 gating flow cytometry panels, we identified immature cell population (SSC^{low}/$CD45^{low}$) and lymphocyte population (SSC^{low}/$CD45^{high}$). We then quantitatively analyzed the expression patterns of 33 cluster differentiation (CD) molecules on individual sample. Finally, we compared the CD expression patterns between AA and hypocellular MDS in both cell populations respectively.

We found that CD19 expression was significantly higher in AA than in hypocellular MDS in both SSC^{low}/$CD45^{low}$ cell population (P=0.001) and SSC^{low}/$CD45^{high}$ cell population (P=0.003). Hypocellular MDS contains significantly higher $CD34^{high}$ cells than AA in SSC^{low}/$CD45^{low}$ populations (mean:28.5% vs 8.5%; range; 1% to 94% vs 2% to 27%; P=0.04). However, patients with both diseases similarly contains very few $CD34^{high}$ cells in SSC^{low}/$CD45^{high}$ cell population (mean: 0.6% vs 2.6%; range: 0.0% to 2% vs 0.0% to 32%; P=0.99).

This study showed that B cells are highly proliferative in both immature stage and mature stage in AA but not in hypocellular MDS; the majority of lymphocyte population are mature cells in both AA and hypocellular MDS. These data indicates that B cells may play a unique role in AA pathogenesis but not in hypocellular MDS; the pathogeneses of both diseases caused by a persistently dysregulated immune microenvironment, not by an acute insult; and CD19 expression pattern may be a useful marker to distinguish AA and hypocellular MDS.

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Chapter 1: Introduction

Aplastic Anemia (AA) and Myelodysplastic Syndrome (MDS) are bone marrow failure diseases. Bone marrow failure occurs in individuals who produce an insufficient amount of red blood cells, white blood cells or platelets.

AA and MDS affect more than 15,000 people in the U.S. every year. The majority of the patients are diagnosed with MDS. It is estimated that about 15,000 people are newly diagnosed with MDS every year[1], and between 600 and 900 people are diagnosed with AA each year. The number of new patients diagnosed seems to be increasing as the population increases and diagnostic technology improvement.

AA and MDS patients usually present with similar but unspecific complaints and symptoms. The common manifestations of these two diseases are fatigue upon exertion, reduced appetite, dizziness, and reduced quality of life, sometimes even cardiopulmonary function compromise. The clinical presentations of those two diseases usually are anemia, thrombocytopenia, or pancytopenia without evidence of blood loss. The social-economic impact of these two bone marrow failure diseases extends way beyond the patients themselves, not only gravely impair patients' personal health, but changing patients and their families' social-economic function as well.

In AA, the normal production of all blood cells reduces because the bone marrow stem cells are damaged. A scarcity of bone marrow stem cells also occurs. Without an accurate amount of blood cells the body struggles to fight off infections and to have accurate blood circulation leading to recurrent infections and cardiopulmonary compromise. Unlike AA, in MDS, a reduction of bone marrow stem cells usually does

not occur, but the stem cells that are present are not normal and in turn do not mature normally. Blood cells and platelets therefore cannot mature correctly. Without enough mature blood cells, anemia will occur.

Because both AA and MDS are caused by bone marrow defects and AA and MDS patients share many common clinical and cytologic features, it is not easy to differentiate AA from MDS in diagnosis. It is especially difficult to diagnose AA from hypocellular MDS. Hypocellular MDS is a subset of MDS in which bone marrow contains much less cells than normal population. AA patients also manifest with hypocellularity in their bone marrow, which makes it particularly difficult to differentiate these two diseases. However, it is crucial to differentially diagnose those two bone marrow failure diseases due to the different treatment options and different prognoses in general.

Currently, diagnosis of AA involves analysis of the peripheral blood and bone marrow as interpreted within the clinical context. A complete blood count with pancytopenia along with a reduction in the number of reticulocytes leads to a diagnosis of AA. An abnormal bone marrow aspiration is a cause for diagnosis of AA if the marrow is hypocellular with a decrease in all elements.

The diagnosis of MDS is based upon findings in the peripheral blood and bone marrow as interpreted within the clinical context. There are three key points that need to be present when diagnosing a patient with MDS. These include unexplained quantitative changes in one or more of the blood and bone marrow elements, morphologic evidence of significant dysplasia and blast forms accounting for less than 20 percent of the total cells of the bone marrow aspirate and peripheral blood.[7]

The current methods described above in measuring which disease is present are unreliable with diagnosing between the two diseases. In fact there is a common misconception that AA is a precursor to MDS due to the number of patients who are misdiagnosed. By looking at protein profiles a protein level difference may be found to aid in diagnosis, making the overall patient diagnosis more reliable. This can be done by finding a protein that is expressed in a different level in patients with one disease versus the other disease.

Because the diagnostic characteristics to tell the difference between hypocellular MDS and AA remains unclear, the mechanism and immunosuppression treatment behind MDS and AA has not been fully developed. Finding a different measurable mechanism in each disease could help with the process of diagnosing between MDS and AA.

Bone marrow failure disease is a consequence of immune system dysregulation. The T lymphocyte system and B lymphocyte system working collaboratively regulate and maintain our human body immune system. Investigating the abnormalities and malfunctions of T lymphocytes and B lymphocytes and their capacity in AA and MDS are significant because investigation may lead to the identity of the mechanisms of these bone marrow failure diseases.

The research question is whether B lymphocytes and T lymphocytes of AA and MDS patients exhibit different protein profiles. My hypothesis is that there is a different profile between AA and MDS because these diseases have different mechanisms.

Chapter 2: Literature Review

Aplastic anemia (AA) and myelodysplastic syndrome (MDS) are disorders that originate in the bone marrow of patients. Bone marrow produces stem cells. When bone marrow is unable to produce red and white blood cells and platelets from its stem cells, patients suffer from a lack of normal blood. Both AA and MDS involve inadequate production of blood cells or platelets. Not enough blood cells can lead to fatigue and infection, while too few platelets can lead to spontaneous or uncontrolled bleeding.[1] This chapter will provide a thorough discussion of aplastic anemia and myelodysplastic syndrome. It will then examine different cytokines involved in aplastic anemia, different cytokines involved in MDS, and the diagnosis between the two diseases.

2.1 Aplastic Anemia

In aplastic anemia, the normal production of all blood cells slows or stops because the bone marrow stem cells are damaged. A shortage of bone marrow stem cells also occurs. Between 600 and 900 people are diagnosed with aplastic anemia each year in the United States.[2] The major causes of aplastic anemia are exposure to a wide variety of drugs and chemicals, ionizing radiation, some viruses, and genetic predispositions. Diagnosing a patient with aplastic anemia can be difficult because a patient often experiences no symptoms in the beginning of the disease progression. A patient with aplastic anemia who is displaying symptoms may be fatigued, have cardiopulmonary compromise, or have recurrent infections.[3]

Diagnosis of AA involves analysis of the peripheral blood and bone marrow as

analyzed within the clinical context. A complete blood count with pancytopenia along with a reduction in the number of reticulocytes leads to a diagnosis of AA. An abnormal bone marrow aspiration is a cause for diagnosis of AA if the marrow is hypocellular with a decrease in all elements.[3]

Aplastic anemia is treated with blood transfusions and immunosuppressant drugs. Adolescents and young adult patients with aplastic anemia require additional attention during diagnosis and treatment due to the likelihood of progression into a more serious form of the illness. The most beneficial treatment method seems to be an HLA-identical sibling cord transplant if available.[4] This seems to be the most beneficial treatment because it has the best chance of completely curing the patient.

2.2 Myelodysplastic Syndrome

Like AA, MDS is a bone marrow failure disease. Further, both AA and MDS have similar symptoms and similar results on diagnostic tests. Unlike AA, in MDS, a shortage of bone marrow stem cells usually does not occur, but the stem cells that are present are not normal and do not mature normally. Blood cells and platelets therefore cannot mature correctly. MDS affects more than 15,000 people in the United States each year.[5] Hypocellular myelodysplastic syndrome is one type of MDS that affects 5-20% of all MDS patients.[6] There are a total of seven subsets of MDS.

MDS occurs most commonly in older male adults with a median age of diagnosis greater than 65 years.[7] MDS has been associated with environmental factors, genetic abnormalities, and other benign hematologic diseases.[7] Like AA, often times a patient will show no symptoms of MDS and the disease will go unnoticed until bloodwork is

performed displaying abnormal blood counts. Anemia is common in patients with MDS and will result in fatigue, weakness, dizziness, cognitive impairment or an altered sense of well-being.[7]

The diagnosis of MDS is based upon findings in the peripheral blood and bone marrow as analyzed within the clinical context. There are three key points that need to be present when diagnosing a patient with MDS. The key points include (a) unexplained quantitative changes in one or more of the blood and bone marrow elements, (b) morphologic evidence of significant dysplasia and (c) blast forms accounting for less than 20 percent of the total cells of the bone marrow aspirate and peripheral blood.[7]

The only cure for MDS is a stem cell transplant. If a stem cell transplant is not possible then the goal switches from curing the disease to relieving symptoms. Treatment consists of different drugs that vary depending on the severity of the disorder and the type of genetic dispositions the patient may or may not have. Chemotherapy is the best option for patients who are in an advanced stage of the disease. While chemotherapy is an aggressive treatment, it is important to treat in MDS patients because MDS has a strong likelihood of progressing into acute leukemia. Chemotherapy could shorten the lives of elderly or extremely ill patients so it is important to have an understanding of the health of the patient before exposing them to toxicity.[8]

In sum, aplastic anemia and myelodysplastic syndrome are bone marrow failure diseases that have very similar symptoms such as fatigue and weakness. Aplastic anemia is a lack of blood cells being produced by the bone marrow while myelodysplastic syndrome is an absence of mature functional blood cells.

2.3 Cytokine profiles in AA

Cytokines are small proteins that are important in cell signaling. Cytokines are released by a broad range of cells including T cells and B cells and affect the behavior of other cells. Behaviors in cells that are affected include immune responses and regulation of maturation, growth, and responsiveness of particular cell populations. It is important to study cytokine profiles in aplastic anemia because it will help determine what causes the disease and how it may be cured.

Most AA cases, aside from cases that are caused by chemicals, radiation or viruses, come about by unknown causes. Mahgroub studied the cause of AA by examining different cytokine gene polymorphisms in patients. He tests whether a higher percent of these cytokine gene polymorphisms actually causes AA. Fifty AA patients were included in his study and his sample was analyzed using a variety of methods including genomic DNA extraction and polymerase chain reaction.

Mahgroub found that there is an increased risk for AA individuals with hyper secretory genotypes compared to those possessing the hypo secretory genotype. There is a statistically significant difference between patients and normal controls regarding the specific polymorphisms in the genes tested, with hyper secretory genotypes higher in AA patients than controls.[9]

Lin examined the role of interferon gamma with the hope that further research on its mechanism could lead to an improved treatment of aplastic anemia and could improve the likelihood of a patient maintaining a disease free status. Interferon gamma is a cytokine. Previous literature before Lin's study thought that autoreactive T lymphocytes

were involved in destroying the hematopoietic stem cells. Using mice, Lin showed that the previously thought idea was not correct. That is, autoreactive T lymphocytes were not involved in destroying the hematopoietic stem cells.

Interferon gamma alone leads to aplastic anemia by disrupting the generation of common myeloid progenitors and lineage differentiation.[10] The inhibitory effect of interferon gamma on hematopoiesis is intrinsic to hematopoietic stem/progenitor cells. The data suggest that AA occurs when interferon gamma inhibits the generation of myeloid progenitors and prevents lineage differentiation, as opposed to infiltration of activated T cells.

In a separate study, Liu evaluated the balance between T-cell immunoglobulin and mucin domain molecules in patients with aplastic anemia. T cell immunoglobulin-mucin proteins are another type of cytokine. T cell immunoglobulin-mucin proteins have been proven by previous literature to be important regulators of immune function. Inaccurate immune functions could lead to the development of aplastic anemia. Liu examined whether there was a specific connection between T cell immunoglobulin-mucin protein levels in normal patients and patients with aplastic anemia.

In Liu's study, samples were taken from patients who were newly diagnosed with aplastic anemia and patients who were in remission, and compared with normal control patients. Samples were analyzed using isolation of RNA from PBMC, enzyme-linked immunosorbent assay, and quantitative real-time polymerase chain reaction analysis. The variety of patient samples used in is the strength of Liu's study. Not only were patients with aplastic anemia analyzed, but also patients in remission were analyzed.

Being the first study examining T cell immunoglobulin-mucin protein levels in patients with aplastic anemia, Liu found that the patients in remission had T cell immunoglobulin-mucin protein levels comparable to the normal control patients. Patients who had aplastic anemia and were not in remission had decreased T cell immunoglobulin-mucin protein levels. This implies that T cell immunoglobulin-mucin protein levels play a role in the development of aplastic anemia.

In sum, prior studies such as Lin, Liu and Mahgroub show that investigating cytokine profiles in patients with aplastic anemia is necessary so that a deeper understanding of the disease mechanism can be developed to improve future treatment and diagnosis.

2.4 Cytokine profiles in MDS

Similar to aplastic anemia, there are many studies examining cytokine profiles in patients with myelodysplastic syndrome. Sashida detailed findings of prior studies that identified different potential mechanisms of bone marrow failure diseases. These mechanisms, which caused alterations to what is found in a healthy person, are the specific reasons cancer is present in patients.[11] Sashida's objective is to understand specific mechanisms of cytokines in hope to understand the development of MDS. Understanding the development of MDS can then find better ways to treat the disease.

Sashida specifically examined how the loss of Ezh2 affects RUNX1 mutants and how the mutant relates to myelodysplastic syndrome developing as a disease. Previous literature displayed that Ezh2 mutations are present when RUNX1 mutations are. Sashida examined the specific cause and effect of Ezh2 loss. The author found that Ezh2 causes

the development RUNX1 mutant-induced MDS. This is important because previously it was known that Ezh2 loss and RUNX1 loss often occurred simultaneously but it was not known that in fact one caused the other.

The loss of Ezh2 causes RUNX1 induced MDS. This proves that the presence of Ezh2 surpresses MDS from forming. Mouse models with Ezh2 loss and RUNX1 expression seemed to develop MDS. Many studies have demonstrated that Ezh2 stops tumors from developing, this study demonstrates the role of Ezh2 in a living organism. This is important because a mouse model is more comparable to a human model than an in vitro model.

New DNA technologies have given an idea to where the problems cause MDS. However the development of MDS is still not clear. Due to the fact that MYD88 is involved in other diseases, Dimicoli tested how it fits into MDS. MYD88 was studied in samples of patients with MDS. This was done in many different ways including bone marrow analysis, RNA analysis, and using an in vitro model. Compared to prior studies, Dimicoli used multiple different sets of data to draw his conclusion.

He found that MYD88 does play a role in the development of MDS and may help in the treatment and diagnosis of the disease. MYD88 is potentially involved in the development of MDS. MYD88 mutations have recently been identified as genetic problems in different diseases. This article provides a deeper look into MYD88 and MDS.[12]

It is difficult to diagnose between myelodysplastic syndrome (MDS) and many other diseases. Some cytokine levels have been found to help aid in diagnosis, however these levels mostly been studied only in western patients. Xiong investigated the cytokine

expression level differences between western and Chinese MDS patients. Many different methods were used to analyze the samples that were obtained from patients with myelodysplastic syndrome. This includes protein array preparation and hybridization, cell cultures, flow cytometry analysis, fluorescence hybridization detection, statistical analysis, expression patterns of cytokines in the bone marrow, immune phenotypic analysis, and chromosome abnormality analysis.[13]

Chinese patients and western patients have different cytokine expression levels because they have a different genetic makeup. By using Chinese patients only in this study new data was developed concerning Chinese MDS patients. Compared with the control group, leukemia inhibitory factor, stem cell factor, stromal cell-derived factor, bone morphogenetic protein 4, hematopoietic stem cell stimulating factor, and transforming growth factor beta in the MDS group were significantly down-regulated while interferon gamma, tumor necrosis factor alpha, and programmed death ligand were significantly upregulated.[13]

There are many different cytokines in patients with MDS and AA, and there are multiple ways to test for them. The method that will be used for further research is described in the next section.

2.5 Diagnosing between AA and MDS

Previous studies have shown that hypocellular or hypoplastic myelodysplastic syndromes share several mechanisms with aplastic anemia. Serio reviewed these mechanisms and discussed how the mechanisms affect the progression and development of MDS. He complied data on information about the overlapping between aplastic

anemia, hypocellular and normo/hypercellular MDS (HMDS) and the immunological derangement in MDS and HMDS. He argued that understanding the immune responses in MDS may produce new immunotherapeutic approaches. Distinguishing HMDS from AA is important because the incidence of progression to acute leukemia is higher in HMDS.[14]

Aplastic anemia and myelodysplastic syndrome with a low blast percentage are often hard to identify and therefore many patients who should be diagnosed as AA are diagnosed as MDS. This leads to the incorrect treatment. Yamazaki provided two different cases and discussed the plan that was put forth to take care of the patient in each case. Yamazaki found that thrombopoieten levels helped determine the best treatment method for bone marrow failure patients because TPO levels seemed to vary between patients with aplastic anemia and myelodysplastic syndrome. This method was interesting because it was able to elaborate on specifics of each patient case. However a caution should be drawn because the sample used to draw the conclusion was so small; a larger study is needed to further evaluate TPO levels in the treatment of MDS.

In conclusion, hypoplastic myelodysplastic syndrome and aplastic anemia share many common clinical and cytologic features. This makes it difficult to diagnosis between these two disorders. It is important to diagnose between the two disorders because there is a higher risk of progression to acute leukemia in patients with MDS than in AA. Because the border between hypoplastic MDS and AA remains unclear, the mechanism and immunosuppression treatment behind MDS and AA has not been fully developed .[15] Finding a different measurable mechanism in each disease could help with the diagnosis process of MDS and AA.

Chapter 3: Materials and Methods

This retrospective study analyzed flow cytometry lymphocytic antigen expression profiles from patients diagnosed as AA and hypocellular MDS as per standard criteria. A total of 31 AA and 26 hypocellular MDS patient cases were recruited. The bone marrow aspirate/biopsy data, bone marrow aspiration flow cytometry reports, and Complete Blood Counts (CBC)s from individual patients were analyzed.

Flow cytometry is commonly used to diagnose blood cancers. A flow cytometry machine can be used to count cells, for biomarker detection, and cell sorting. Cell size and volume can be determined by a flow cytometry machine in addition to an analysis of the cell surface and intramolecular molecules.

In order to begin using the flow cytometer the mononuclear cells must be obtained from the bone marrow. This process is done by diluting, filtering and centrifuging the cells in a carefully established procedure. Once mononuclear cells are obtained from the human bone marrow aspirates, a staining procedure is used and the cell is then incubated with labeled fluorochrome-labeled antibodes.

When the stained, suspended cells are run through the flow cytometer, the hydrodynamic focusing of the flow cytometer allows the cells to pass through the machine in a single file fashion. A laser light source allows forward and side scattered light from all cells to be detected. Forward scatter determines cell size and side scatter is proportional to the granularity of the cells. Many times cell populations can distinguished from the side scatter and forward scatter alone.

When running blood samples on the flow cytometer granulocyte cell populations have a high SS and FS. Monocytes have a high FS but lower SS due to their large size but

less granular nature. Lymphocytes and immature cells produce less FS and a low SS because they are smaller and not granular. Lymphocytes and immature lymphoblast cells also have different characteristics as far as size and granularity. It is in this way that we were able to determine the amount of lymphocytes vs immature cell population in our flow cytometry data. Figure 1 displays how flow cytometry results appear and are categorized.

Immature cells are the precursor to lymphocyte cells and are found in different amounts in different diseases due to the fact that immature cells may proliferate at an unusual uncontrollable rate depending on the disease.

There is also a fluorescence emitted from the stained cells that are detected. This is used to determine whether the cells express a particular protein. The protein of interest is stained with a fluorescent antibody. The fluorescent antibody emits light when it is excited by a laser with the corresponding excitation wavelength. The fluorescent stained cells or particles can be detected individually thus detecting the target proteins. Target proteins in this experiment were CD19 and CD34. It is in this way that the 33 cluster differentiation expression patterns on the molecules were investigated.

Using side scatter (SSC) vs. CD45 gating flow cytometry panels, we identified immature cell population ($SSC^{low}/CD45^{low}$) and lymphocyte population ($SSC^{low}/CD45^{high}$). We then quantitatively analyzed the expression patterns of 33 cluster differentiation (CD) molecules on individual sample. Finally, we compared the CD expression patterns between AA and hypocellular MDS in both cell populations respectively.

Chapter 4: Results and Discussion

This experiment involved the analysis of two different populations of cells, an immature cell population and a mature lymphocyte population. Immature cell population was defined as $SSC^{low}/CD45^{low}$ using flow cytometry as shown in Figure 1. Lymphocyte cells were defined as $SSC^{low}/CD45^{high}$ using flow cytometry as seen in Figure 1. This flow cytometry data was then analyzed to count the number of cells that are immature cells and lymphocyte cells. The flow cytometry data was also used to measure expression patterns of CD19 and CD34 in these immature and lymphocyte cells.

The first comparison was done between immature ($SC^{low}/CD45^{low}$) AA and MDS cells and CD34 expression. When CD34 expression patterns were compared between AA (N=16) and hypocellular MDS (N=18) in $SSC^{low}/CD45^{low}$ cell population, CD34 expression was significantly higher (p = 0.02) in hypocellular MDS (mean =11.8) than in AA samples (mean = 31.4) [Table 2].

This CD34 expression pattern was also compared within mature lymphocyte ($SC^{low}/CD45^{high}$) MDS and AA cells. When CD34 expression patterns were compared between AA (N=24) and hypocellular MDS (N=17) in $SC^{low}/CD45^{high}$ cell population, CD34 expression was not statistically significant (p = 0.99) in hypocellular MDS (mean = 2.6) than in AA samples (mean =0.6) [Table 3].

In conclusion, hypocellular MDS contains significantly higher $CD34^{high}$ cells than AA in $SSC^{low}/CD45^{low}$ populations (mean:28.5% vs 8.5%; range; 1% to 94% vs 2% to 27%; P=0.04). However, patients with both diseases similarly contains very few $CD34^{high}$ cells in $SSC^{low}/CD45^{high}$ cell population (mean: 0.6% vs 2.6%; range: 0.0% to 2% vs 0.0% to 32%; P=0.99).

Immature and mature AA and MDS populations were also analyzed with CD19 expression. When CD19 expression patterns were compared between AA (N=15) and hypocellular MDS (N=21) in SSC$_{low}$/CD45$_{low}$ cell population, CD19 expression was significantly higher (p = 0.001) in AA (mean =29.9) than in MDS samples (mean =11.4) [Table 4]. When CD19 expression patterns were compared between AA (N=30) and hypocellular MDS (N=21) in SSC$_{low}$/CD45$_{high}$ cell population, CD19 expression was significantly higher (p = 0.003) in hypocellular MDS (mean =11.4) than in AA samples (mean = 29.9) [Table 5].

Therefore CD19 expression was significantly higher in AA than in hypocellular MDS in both SSC$_{low}$/CD45$_{low}$ cell population (P=0.001) and SSC$_{low}$/CD45$_{high}$ cell population (P=0.003).

Patient characteristics are defined in Table 1 and have an analysis of different characteristics comparing patient AA and hypocellular MDS populations. The characteristics included in this analysis are age, sex, cellularity, transformation, transfusion dependence, red blood cells, white blood cells, hemoglobin, hematocrit, platelets, lymphocytes, blasts, and lactate dehydrogenase.
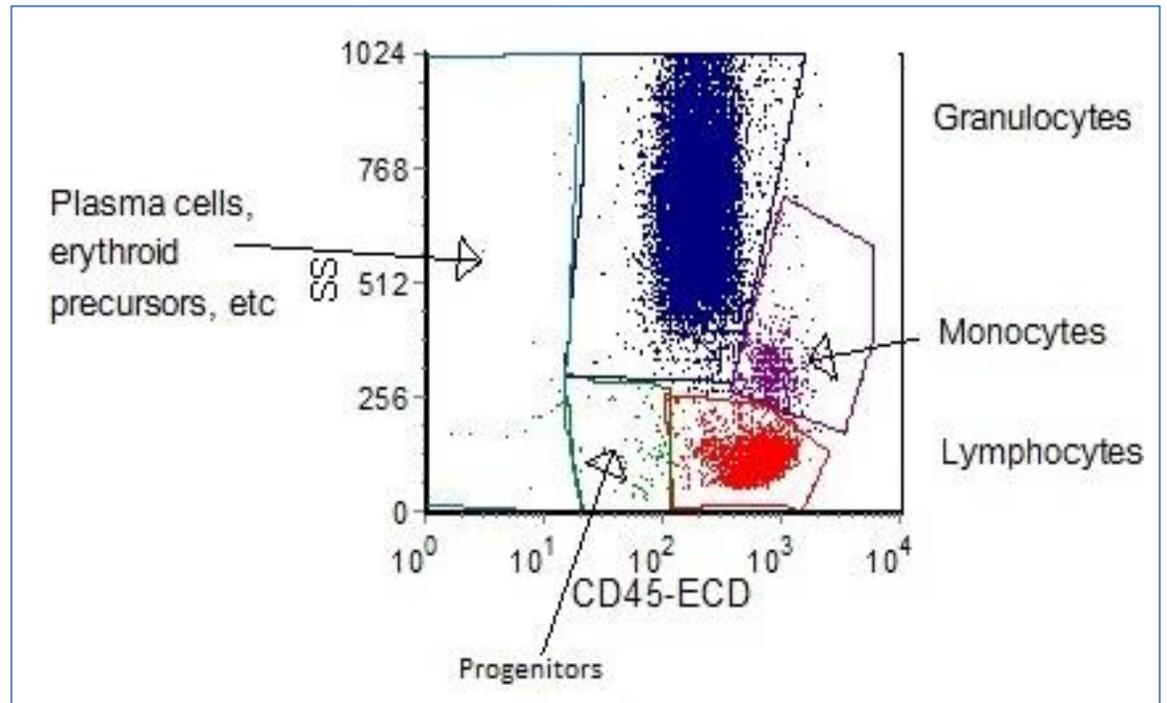
Figure 1: Flow cytometry population localization using SSC. Vs CD45.

Immature cell population was defined as SSC^{low}/CD45^{low}. Lymphocyte cells

were defined as SSC^{low}/CD45^{high}.

Table 1:	Patient Characteristics	
	AA	**Hypocellular MDS**
N	31	26
Age	Range (2-87): Mean 45.2	Range (6-80); Mean 58.1
Sex		
Male	41.94%	46.15%
Female	58.1%	53.85%
Cellularity		
Hypocellular	100%	69.23%
Normocellular	0%	30.80%
Transformation		
	9.68% MDS	7.69% From AA
		7.69% AML
Transfusion dependent	32.26%	61.54%
RBC		
Normal	4.2-6.7 million cells/mcL	
	3.64±16.44	9.46±0.45
WBC		
Normal	4.5-10.0 cells/mcL	
	2.81±2.76	3.63±3.76
HGB		
Normal	12.1-17.2 gm/dL	
	3.67±4.39	3.46±1.42
HCT		
Normal	36.1-50.3 percent	
	27.56±12.01	28.0±4.39
PLT		
Normal	150-400 thousand platelets/mcL	

	27.51±27.12	124.77±167.66
Lymphocytes		
Normal	**25-40 percent**	
	64.88±23.93	49.07±23.04
Blast		
Normal	**0 percent**	
	0.05±0.19	0.46±2.35
LDH		
Normal	**313-618 unit/L**	
	531.65 (282-730)	750.24 (253-2681)

Comparison of CD34 Expression in SSC^{low}/CD45^{low} cell population (P=0.02)					
	N	min	Max	Mean	S
AA	16	3.0	27.0.	11.8	7.6
MDS	18	6.0	94.0	31.4	30.5

Table 2: Comparing CD34 expression patterns between AA and hypocellular MDS in SSC^{low}/CD45^{low} cell population. CD34 expression was significantly higher in hypocellular MDS than in AA in SSC^{low}/CD45^{low} cell population (P=0.02).

Comparison of CD34 Expression in SSC^{low}/CD45^{high} cell population (P=0.99)					
	N	Min	Max	Mean	S
AA	24	0	32.0	2.6	6.9
MDS	17	0	2.0	0.6	0.7

Table 3: Comparing CD34 expression patterns between AA and hypocellular MDS in SSC^{low}/CD45^{high} cell population. CD34 expression were similarly low in both hypocellular MDS and AA in SSC^{low}/CD45^{high} cell population (P=0.99).

Comparison of CD19 Expression in SSC^{low}/CD45^{low} cell population (P=0.001)				
	N	min	Max	Mean	S
AA	15	3.0	78	29.9	20.8
MDS	21	0.0	49	11.4	13.2

Table 4: Comparing CD19 expression patterns between AA and hypocellular MDS in SSC^{low}/CD45^{low} cell population. CD19 expression was significantly higher in AA than in hypocellular MDS in SSC^{low}/CD45^{low} cell population (P=0.001).

Comparison of CD19 Expression in SSC^{low}/CD45^{high} cell population (P=0.003)				
	N	Min	Max	Mean	S
AA	30	0	62	12.9	16.9
MDS	21	0	22	5.9	8.4

Table 5: Comparing CD19 expression patterns between AA and hypocellular MDS in SSClow/CD45low cell population. CD19 expression was significantly higher in AA than in hypocellular MDS in SSC^{low}/CD45^{high} cell population (P=0.003).

Chapter 5: Conclusion

The goal of this thesis was to determine a different measurable mechanism in both MDS and AA which could help with the diagnosis process of MDS and AA. This was accomplished through the retrospective analysis of the flow cytometry data.

Analysis of flow cytometry results concerning markers CD34 and CD19 enabled conclusions to be drawn about the data. Cluster of differentiation 34, also known as CD34, is used a marker in this study because the protein encoded by this gene may play a role in the attachment of stem cells to the bone marrow extracellular matrix. This single-pass membrane protein is highly glycosylated and phosphorylated by protein kinase C.[17] Cluster of differentiation 19, also known as CD19, is used as a marker in this study because it is found on the surface of B cells. The CD19 gene encodes a cell surface molecule that assembles with the antigen receptor of B lymphocytes in order to decrease the threshold for antigen receptor-dependent stimulation.[16]

Results from our study concerning stem/progenitor cell population and mature cell population as quantified by CD34 expression patterns suggest that hypocellular MDS contains higher amounts of stem/progenitor cell population when compared to AA; however, both hypocellular MDS and AA contain a comparable amount of mature lymphocyte cells.

In AA, B cells are highly proliferative in both immature stage and mature stage. This data indicates that B cells may play a unique role in AA pathogenesis but not in hypocellular MDS. In both AA and hypocellular MDS, the majority of lymphocyte

population are mature cells. This data suggests that the pathogeneses of both diseases is caused by a persistently dysregulated immune microenvironment, not by an acute insult.

Our study also indicates that CD19 expression was higher in AA samples when compared to MDS samples of both immature and mature cell populations. CD19 expression pattern may be a useful marker to distinguish AA and hypocellular MDS.

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