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Atypical Localization of Malignant Plasma Cells in Non-Viable Cell Area on Flow Cytometry Light-Scatter Dot Plot

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ADEF 1 **Tajana Juranovic**
ABCD 2 **Gary Harvey**
BCD 2 **Jennie Johnson**
BDEF 3 **Deng Zhang**
ABDE 2 **Milton J. Plata**
ACDE 2 **Oscar C. Estalilla**
ABDEF 2 **Tomislav M. Jelic**

1 Department of Internal Medicine, University Hospital Centre (KBC Rebro), Zagreb, Croatia
2 Department of Pathology and Laboratory Medicine, Charleston Area Medical Center, Charleston, WV, U.S.A.
3 Cancer Center, Charleston Area Medical Center, Charleston, WV, U.S.A.

Corresponding Author: Tomislav M. Jelic, e-mail: tjelic@suddenlink.net
Conflict of interest: None declared

Case series

Patient: Male, 60 • Male, 50
Final Diagnosis: Multiple myeloma
Symptoms: Back pain
Medication: —
Clinical Procedure: Bone marrow core biopsy and aspirate
Specialty: Hematology

Objective: Rare co-existence of disease or pathology

Background: Multi-parameter (multicolor) flow cytometric study of the bone marrow aspirate is a very useful tool for diagnosis of plasma cell dyscrasia and for evaluation of post-therapy bone marrow for minimal residual disease.

Case Report: We present a case of a 50-year-old man with multiple myeloma, whose plasma cells on a bone marrow aspirate flow cytometric study showed atypical placement on a light-scatter dot plot, both on forward and side scatter. The bone marrow aspirate sample was 33 hours and 11 minutes old, and the light-scatter dot plot demonstrated that plasma cells, detected by their expression of CD138, CD38, and CD56, occupied an area otherwise characteristic for dead cells and cell detritus. Expressions of CD138 and CD56 were dim (down-regulated).

Conclusions: Morphologically atypical plasma cells with irregular nuclear contours/polylobated nuclei from non-fresh samples can present with atypical localization in the area of dead cells. Our study of the multiple myeloma patient with normal localization of plasma cells on a light-scatter dot plot showed a fraction of plasma cells in the dead cell area with dim expression of CD138 and CD56, suggesting that plasma cells may deteriorate (age) rather rapidly, losing surface markers even in less than 24-hour-old specimens. We suggest that the non-viable cell/dead cell area should be checked for expression of CD138 so as not to miss plasma cell dyscrasia, especially if the specimen was run 24 hours after bone marrow sampling.

MeSH Keywords: Flow Cytometry • Multiple Myeloma • Syndecan-1 • Tissue Survival

Full-text PDF: <https://www.amjcaserep.com/abstract/index/idArt/910661>

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Background

The diagnosis of multiple myeloma is established by judicious interpretation of clinical symptoms and signs, laboratory findings including complete blood count, peripheral blood smear examination, serum and urine protein electrophoresis and immunofixation electrophoresis, chemistry screen for calcium, creatinine, beta-2 microglobulin, C-reactive protein, lactate dehydrogenase, erythrocyte sedimentation rate, imaging study of the bones, bone marrow biopsy and aspirate with chromosomal study, and FISH studies (fluorescence *in situ* hybridization) with probes for standard multiple myeloma translocations. Flow cytometric analysis of the bone marrow aspirate is also a very useful diagnostic contributor; it can detect clonal (monoclonal intracytoplasmic light chains) and immune-phenotypically aberrant plasma cells and thus may be useful in establishing diagnosis and could be a powerful tool for the detection of minimal residual disease following treatment [1].

Normal plasma cells on the light-scatter dot plot are stretched along the forward axis and side scatter axis, while malignant plasma cells are somewhat less dispersed (more clustered) and, in comparison with lymphocytes, usually fall much more forward on forward scatter and slightly forward on side scatter.

Normal plasma cells strongly express CD38 and CD138, and both markers are very useful in delineating (gating) plasma cells. The most specific plasma cell marker is CD138, but it can be positive in some lymphomas and in a variety of carcinomas [2]. Normal plasma cells also express CD19, CD79a, CD27, CD45, and polyclonal intracytoplasmic immunoglobulins. Normal plasma cells do not express CD20, CD200, CD117, or CD28. Malignant plasma cells also strongly express CD38 and CD138, but the expression of CD138 tends to be brighter and CD38 dimmer than in normal plasma cells [3]. Malignant plasma cells express monoclonal intracytoplasmic immunoglobulins.

Malignant plasma cells may aberrantly express CD56, CD28, CD117, CD20, CD200, CD52, and CD10, while CD19, CD27, or CD45 are commonly lost or diminished [1]. The bcl-1 (cyclin D1)

is expressed in cases with a translocation t(11;14)(q13;q32) and in some myelomas with hyperdiploidy [4].

The percentage of plasma cells obtained by flow cytometric analysis of the bone marrow aspirate is usually 60% to 70% lower than the percentage of plasma cells obtained by manual count on bone marrow aspirate smear [1,5]. This is mainly due to the technical aspect of the bone marrow biopsy and aspiration. The first aspirate is usually used for making a bone marrow aspirate smear. The second and/or third aspirate for flow cytometry are usually taken from the same site, which, in comparison to the first aspirate, would usually be depleted of marrow cells and diluted by peripheral blood. Plasma cells are fragile and thus are also lost during the processing steps (e.g., centrifugation) for flow cytometry [5]; consequently, flow cytometry is not useful for quantifying plasma cells in the bone marrow at diagnosis [5]. However, despite this obstacle, the multi-parameter (multicolor) flow cytometric method is very sensitive (sensitivity 10^{-4}), is more sensitive than immunohistochemistry (sensitivity 10^{-2} – 10^{-3}) and can detect a very small number of malignant plasma cells (1 malignant plasma cell among 10 000 normal hematopoietic cells) [6]. Therefore, multicolor flow cytometry is a very useful tool for detecting minimal residual disease following treatment of myeloma, especially after autologous stem cell transplantation [7]. We need to keep in mind that malignant plasma cells in the bone marrow are placed focally in a “patchy” pattern and thus might be missed during sampling for flow cytometry, as well as on an immunoperoxidase study, giving a falsely negative result in patients with plasma cell dyscrasia.

Case Report

Pertinent laboratory parameters for 2 patients with multiple myeloma (a 60-year-old man with an expected location of plasma cells and a 50-year-old man with a location of plasma cells in dead cell area) are presented in Table 1. Microscopic images of plasma cells of the patient with their location in dead cell area are presented in Figure 1. Dot plots of the patient with plasma cells located in an expected area are presented

Table 1. Pertinent laboratory parameters of the 2 multiple myeloma patients.

	Age years	WBC× 10 ³ /mm ³	RBC× 10 ⁶ /mm ³	Hgb (g/dl)	MCV (fL)	HTC (%)	MCHC (g/dl)	RDW (%)	Plt× 10 ³ /mm ³	MS (g/dl)	Plasma (%)
NP	60	9.7	2.9	8.7	89.5	25.9	33.5	16	178	5.8	18.3
Pt	50	13.6	3.62	11.7	90.9	32.9%	35.5	15.5%	174	2.2	20

NP – patient with multiple myeloma with standard location of plasma cells (“normal” multiple myeloma patient); Pt – patient with atypical localization of malignant plasma cells; WBC – white blood cells; RBC – red blood cells; Hgb – hemoglobin; MCV – mean corpuscular volume; HTC – hematocrit; MCHC – mean corpuscular hemoglobin concentration; RDW – red blood cell distribution width; Plt – platelets; MS – monoclonal spike; plasma (%) – percentage of plasma cells obtained by manual count on bone marrow aspirate smear.

Figure 1. Patient with atypical localization of plasma cells in the dead cell/detritus area. Bone marrow core biopsy stained with hematoxylin and eosin and with immunoperoxidase stains for CD138, cyclin D1 (Bcl-1), and immunoglobulin light chains lambda and kappa and Wright/Giemsa stained bone marrow aspirate smear.

in Figure 2. Testing was run 22 hours after bone marrow aspiration. Dot plots of the patient with all plasma cells located in the dead cell area are presented in Figure 3. Testing was run 33 hours and 11 minutes after collection of the specimen.

Hematoxylin/eosin and immunoperoxidase stained sections of the bone marrow core biopsy (Figure 1, the first image in the upper row) of the patient with atypically located plasma cells demonstrated a sheet of monoclonal immunoglobulin lambda light chain restricted plasma cells that strongly expressed CD138 and lambda and were negative for kappa and cyclin D1. The malignant plasma cells strongly expressed CD138, CD38, and CD56, and did not express CD19, CD45, CD20, or CD10. The malignant plasma cells did not express proliferation marker Ki-67 (0%).

The Wright-Giemsa-stained bone marrow aspirate smear demonstrated plasma cells with irregular nuclear contours and lobulated nuclei (Figure 1, the third picture in the first row). The plasma cells were not large, about 12 mm in diameter, slightly larger than small lymphocytes, slightly smaller than neutrophils, and smaller than the average size of multiple myeloma cells. (Figure 1). The irregularity of the nuclei of plasma cells can be easily noted on negative immunoperoxidase stain for Bcl-1 (Figure 1).

Plasma cells are located in the dead cell/detritus area, and expression of both CD138 and CD56 is dim. The specimen was run 33 hours and 11 minutes after its collection.

Dot plots demonstrated that, in comparison to standard multiple myeloma plasma cells, plasma cells of our 50-year-old patient displayed atypical localization in the area where we expect non-viable (dead) cells (Figure 3, the second image). Midline of the plasma cell cloud on forward scatter was at division 2.2 with cloud spanning from division 1.5 to division 3.3. In comparison, midline of the plasma cell cloud of our prototypic multiple myeloma patient (Figure 2, the first row, second image) on the forward scatter was at division 8 with the bottom at division 6 and top outside the depicted forward scatter axis (Table 2).

On the side scatter, the midline of the cloud of atypical plasma cells was at division 2.7, with a range from division 1.8 to 3.6. The midline of the cloud of standard multiple myeloma cells was at division 1.6, with a range from division 0.6 to division 2.7. Dot plots in the second row of the patient with normally located plasma cells revealed a small population of myeloma cells in the dead cells/detritus area (Figure 2). The midline of the cloud on forward scatter was at division 2.5, bottom at division 1, and top at the division 4. On the side scatter midline of the cloud was at division 2, with a range from division 1.5 to division 2.8. In comparison with normally located myeloma cells, midline of the cloud of myeloma cells with reduced viability decreased on forward scatter for 5.5 divisions, with no significant change on side scatter. The first column indicates that expression of CD138 and expression of CD56 were dim (down-regulated) in plasma cells located in the dead cell area.

Figure 2. Dot plots (the first row) from the patient with multiple myeloma with standard location of plasma cells. Plasma cells located in the dead cell area from the same patient showing down-regulation of CD138 and CD56 (the second row). The specimen was run 22 hours after its collection.

The patient preferred to postpone bone marrow transplantation for now and responded well to chemotherapy with lenalidomide + bortezomib + dexamethasone with the free light chains back to normal and the disappearance of the monoclonal spike on serum protein electrophoresis.

Discussion

Multiparametric flow cytometric analysis of the bone marrow aspirate is a very useful adjunct in establishing a diagnosis of

plasma cell dyscrasia and investigation for residual plasma cells after therapy. Benign plasma cells on light-scatter plots fall in unpredictable areas, almost randomly scattered along length of forward scatter axis and length of side scatter axis. In other words, normal plasma cells unlike other bone marrow hematopoietic cells do not form light-scatter-defined cluster [3]. This might be due to the variation in size of normal plasma cells (forward scatter) and the variation in granular content (immunoglobulins) among the plasma cells (side scatter). In multiple myelomas, malignant plasma cells usually form tighter light-scatter clusters of cells than benign plasma cells, but

Figure 3. Dot plots from the patient with multiple myeloma with atypical location of plasma cells.

Table 2. Parameters (coordinates of plasma cell cloud in divisions) from forward scatter/side scatter dot plots.

	FS	FS r	SS	SS r
NP	8	>6	1.6	0.6–2.7
NP	2.5	1–4	2	1.5–2.8
Pt	2.2	1.5–3.3	2.7	1.8–3.6

FS – midline of the plasma cell cloud on forward scatter; FS r – range of plasma cell cloud on forward scatter; SS – midline of the plasma cell cloud on side scatter; SS r – range of plasma cell cloud on side scatter. NP – top row presents parameters from the second image in the first row in Figure 2. (Multiple myeloma patient with plasma cells located in an expected area); NP – middle row presents parameters from the second image in the second row in Figure 2. (Multiple myeloma patient with plasma cells located in an expected area and with a small population of plasma cells located in a dead cell area); Pt – bottom row presents parameters from the second image in Figure 3. (Multiple myeloma patient with plasma cells located in a dead cell area).

not as tight as benign or malignant lymphocytes do. In our flow cytometry laboratory, on the light-scatter dot plot, malignant plasma cells are usually located far forward on the forward scatter axis so that the midline of the malignant plasma cell cloud is at division 8, with the bottom of the cloud at division 6 and the top of the cloud exceeding the depicted forward scatter axis. On the side scatter, malignant plasma cells are less dispersed, with the majority of them at division 1.6, with a range from division 0.6 to division 2.7 (Figure 2, the second picture on top, forward scatter/side scatter dot plot). Our 50-year-old patient displayed atypical localization of malignant plasma cells. His plasma cells were located in the non-viable cell area (dead cell area). They were present 5.8 divisions away (down) from standard placement of malignant plasma cells on forward scatter and 1.1 divisions away up on side scatter. This quantification should be taken “cum grano salis” (not as absolutely accurate) since the patient with atypically localized plasma cells was analyzed on the Beckman Coulter FC-500

Flow Cytometer, while the patient with normally placed plasma cells was analyzed on the Beckman Coulter Navios Flow Cytometer. These 2 analyzers, being optimized and calibrated to different voltages and different optimal settings, cause a slight difference in dot plot placement of cells along the x and y axis. Although difference in divisions cannot be accurately quantified, it is obvious that the difference in the location of plasma cells is striking. Atypically placed plasma cells are to the naked eye much lower on forward scatter than normally placed multiple myeloma cells and obviously placed in dead cell/detritus area. We presume that the side scatter displacement was due to the prominent irregularity of the nuclear contours. Atypically placed plasma cells were not dead cells. Rocket pattern of dead cells is not present on CD138/CD56 dot plot in Figure 3, and there was no evidence of rocket pattern of nonspecific staining of detritus/dead cells with all other used markers (antibodies) for flow cytometric study, as demonstrated by gating the plasma cells in the dead cell

area. Unfortunately, a 7AAD viability dye was not analyzed with this sample. Plasma cells in the bone marrow core biopsy were also fully alive and stained by immunoperoxidase technique only with CD138 and lambda and not with other antibodies. Negativity for cyclin D1 and kappa is presented in Figure 1. Gating of the dead cell area in a patient with typically placed plasma cells demonstrated an additional small but distinct population of CD138-, CD38-, and CD56-positive plasma cells. The middle of the cloud of myeloma cells in the dead cell area was 5.5 divisions below the middle of the cloud of normally placed myeloma cells. Plasma cells in this dead cell area displayed CD138 and CD38 with slightly dimmer expression than normally placed plasma cells. In comparison with normally placed plasma cells in the same figure, the CD138/CD56 cloud of plasma cells in the dead cell area moved down along both axis. In other words, these markers become down-regulated, which can be a sign of aging of cells in the sample tube with the associated loss of surface markers. The presence of normally placed plasma cells and the presence of plasma cells in the dead cell area suggest a mixture of fully viable plasma cells and less viable plasma cells. The gradual decrease in the expression of CD138 and CD56 from bright in normally located plasma cells to dimmer in plasma cells in the dead cell area suggests rapid aging of plasma cells even in the freshly sampled tube (First column Figure 2). This fact may partially explain the long stretch of plasma cells along the forward scatter axis and side scatter axis.

The bone marrow sample of our patient with atypical localization of all plasma cells was 33 hours and 11 minutes old when run through the flow cytometry instrument. This is beyond the recommended time of 24 hours for an ACD (acid-citric-glucose)

anticoagulated specimen. As expected, the decreased viability of cells with associated loss of surface markers is in accordance with the dim expression of CD138 and CD56, which was dimmer in the specimen 33 hours and 11 minutes old than in 22-hour-old specimen (Figures 2, 3). We found that plasma cells in the sample tube age rapidly and lose surface markers with associated down-regulation (dim expression) of CD138 and CD56, and myeloma cells dislocate to the dead cell area.

We presume that the forward scatter displacement of the plasma cell cloud to the dead cell area was for the most part due to the decreased viability of myeloma cells and was minimally or not at all due to their smaller than average size.

Conclusions

Malignant plasma cells on the light-scatter dot plot can be atypically located in the dead cell area and thus not recognized as such. Multiple myeloma cells in the sampling tube deteriorate rather fast, lose their markers, exhibit down-regulation of CD138 and CD56, and become displaced to the dead cell/detritus area.

We suggest that the non-viable cell area be checked for expression of CD138, especially in an older specimen, so as not to miss the presence of plasma cell dyscrasia.

Conflict of interest

None.

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