

CD38 expression by plasma cells in extramedullary multiple myeloma

Extramedullary disease (EMD) is a rare manifestation of multiple myeloma (MM) characterized by a proliferation of plasma cells (PC) outside of the bone marrow (BM), and it represents an unmet medical need in the treatment of MM patients (pts). Thus, a better characterization of PC features of the EMD is critical to improve the treatment.

EMD can be found at the time of diagnosis (primary EMD) with an estimated incidence of 3–5% or at the time of relapse (secondary EMD) with an incidence of 6–20%.¹ Moreover, EMD can be divided into two groups: EMD bone-related (EM-B), in which PC extend directly from osteolytic bone lesions, and EMD soft tissue-related (EM-S) that results from PC infiltration into soft tissues with no relationship to the bone. EM-B cells are still partially dependent on the BM microenvironment, while EM-S cells carry different biological characteristics.² The outcome is worse for pts with EM-S compared with those with EM-B and this difference could be due to the different biological features of the EM PC.³

The typical sites of EM-S may be different according to the stage of MM. At diagnosis, EMD is typically found in the skin and soft tissues, with usually only one site involved.^{1,4} At relapse, EMD can appear in more unusual sites such as the liver, kidneys, lymph nodes, breast, pleura, pericardium, and the central nervous system.^{1,3} The presence of EM-S represents an aggressive form of MM linked to high-risk genetic features, increased proliferation, and resistance to therapies.^{2–4}

Previous studies have shown that 1q21 amplification (amp(1q21)), a high-risk chromosomal abnormality, occurs more frequently in pts with EMD.⁵ It was shown that the frequency of amp(1q21) is higher in EM lesions than in BM. Therefore, it is possible that amp(1q21) is a contributing factor to EMD development. It is also noted that the increase in EMD amp(1q21) copy number negatively affected both progression-free survival (PFS) and overall survival (OS).⁵

Beyond genetic alteration, EM PC also present a modulation of different adhesion molecules involved in BM homing, which supports clonal PC migration through the bloodstream.^{6,7} EM PC demonstrate downregulation of adhesion glycoprotein CD56, which normally maintains the connection of MM cells to the BM osteoblastic niche.^{6,7} EM PC also demonstrate an upregulation of CD44, which is a membrane glycoprotein involved with cell homing and recirculation. The CD44v isoform is commonly found in cancer stem cells and has low-level expression in normal cells.⁷

Another important adhesion molecule in the MM biology is CD38: a multifunctional transmembrane glycoprotein, highly expressed by both normal and malignant PC. CD38 is also an ectoenzyme involved in the production of adenosine in

the BM niche.⁸ CD38 is considered a hallmark of MM cells and a therapeutic target for an anti-CD38 antibody-based approach. However, there is limited data regarding the efficacy of daratumumab, an anti-CD38 monoclonal antibody, in EMD.³ Clinical trials have demonstrated that daratumumab is less effective in EMD pts compared to MM pts without EMD.⁹ This phenomenon could be explained by decreased CD38 expression on EMD PC.^{10,11}

The CD38 expression profile of PC in EMD pts is still unknown thus in this study, we performed a comparative analysis of CD38 expression and other adhesion molecules such as CD56 and CD44 between BM PC and EMD PC. The study was approved by the local Ethic Committee and conducted according to the Declaration of Helsinki. Pts included in the study signed a written informed consent.

We retrospectively investigated 22 MM pts with biopsy-proven EMD treated in our hematological unit at the University of Parma from 1999 to 2020. The immunostaining from BM and EMD biopsies were scored on a 5-tiered scale using a semi-quantitative evaluation of the percentage of CD38, CD56, and CD44 expressed by MM cells.

In our cohort of MM pts, three pts presented with EMD at diagnosis, while 19 pts presented EMD at relapse. Forty six percent of the pts presented with a high-stage MM (International Staging System [ISS] III). Twenty pts presented an initial diagnosis of MM, while two pts were affected by primary PC leukemia (pPCL). The median age at the time of the diagnosis was 67 years old (range, 47–76). Men made up 60% of the entire population. The median time to EMD appearance during relapse was 29 months (range, 9–201 months). The most frequent MM subtype was light chain MM (41%), followed by immunoglobulin (Ig)G and IgA. A median IgG lactate dehydrogenase value, available in 14 pts, was slightly elevated (517 U/L, normal value <500 U/L) (Table 1).

Table 1. Patient characteristics at diagnosis.

Characteristics	N=22
Sex: M/F (%)	13/9 (60/40)
Median age in years (range)	67 (47-76)
Diagnosis: MM/pPCL (%)	20/2 (91/9)
EMD-S: diagnosis/relapse (%)	3/19 (14/86)
Isotype: IgG/IgA/LC (%)	6/7/9 (27/32/41)
Light chain: κ/λ (%)	10/12 (45/55)
ISS: I/II/III (%)	6/6/10 (27/27/46)

M: male; F: female; MM: multiple myeloma; pPCL: primary plasma cell leukemia; EMD-S: extramedullary disease soft tissue-related; Ig: immunoglobulin; LC: light chain; ISS: International Staging System.

The most frequent CRAB criteria were anemia (73%), followed by bone disease (64%), renal failure (27%), and hypercalcemia (14%). In our population, a high BM tumor burden was documented with a median BM PC infiltration equal to 60% (range, 0-90%).

Overall, 15 of 18 (88%) pts with available *in situ* hybridization (FISH), showed two or more cytogenetic alterations. The most frequent cytogenetic aberration reported was amp(1q21) (74%), as reported by the literature, followed by del(13q) (58%), del(1p32) (42%), and hyperdiploidy (21%) (Online Supplementary Figure S1).

Fifty-five percent of MM pts developed multiple plasmacytomas. The most common sites were soft tissue and the liver/spleen, which represent 42% of the total EMD, followed by lymph nodes (15%). In 41%, the EMD relapse was dissociated from the BM relapse.

Furthermore, our immunohistochemistry analysis showed a high score of CD56 (3-4) in five of 18 (28%) EMD samples and was absent in 12 of 18 (67%) pts. Discordant expression of CD56 was observed in 17% of samples, with a strong downregulation in the EMD samples compared to BM. CD44 showed a high score in ten of 16 (63%) EMD samples and was absent in three of 16 (19%). Four pts (25%) showed an upregulation of CD44 expression in the EMD samples compared to the BM (Figure 1). Moreover, the expression of CD38 had a high score in 15 of 19 BM samples (79%) and was absent in three of 19 (16%) EMD samples. A downregulation of CD38 expression was observed in 26% of the pts with in the EMD samples

compared to BM. Indeed, 42% of total EM samples were characterized by a low CD38 immunohistochemical score with a percentage of positive PC inferior to 49%. Noteworthy, in about a quarter of pts with matched BM and EMD biopsies available, a reduction of CD38 expression on neoplastic PC was observed in EM samples compared to BM (Figure 2).

This observation confirms that CD38 median fluorescence intensity (MFI) is higher in BM PC compared to circulating tumor cells, which are characterized by a weak dependence on the BM microenvironment and a tendency to egress in peripheral blood.¹² Moreover, we have shown that pts with a low EMD immunohistochemical score in EM had lower baseline CD38 expression levels on BM PC compared with pts with a high immunohistochemical score (mean log₁₀ MFI CD38: 4,3 vs. 3,7; *P*=0.004). In addition, we evaluated the OS in the cohort of MM pts according to the CD38 immunohistochemical score previously used in EMD and BM samples. We found that pts with EMD and a low CD38 score have worse OS compared to pts with a high CD38 score (7,3 vs. 18,05 months; *P*=0.039). This observation is in line with another study that showed how a CD38-low CD45⁺ CD81⁺ phenotypic profile identified a group of MM pts with poor outcomes.¹³

Regarding the treatment of this cohort of pts, 20 of them received active treatment for EMD. Two pts did not receive active treatment for EMD because they had poor performance status. Radiation therapy as local therapy was delivered to two pts mainly with a disease control

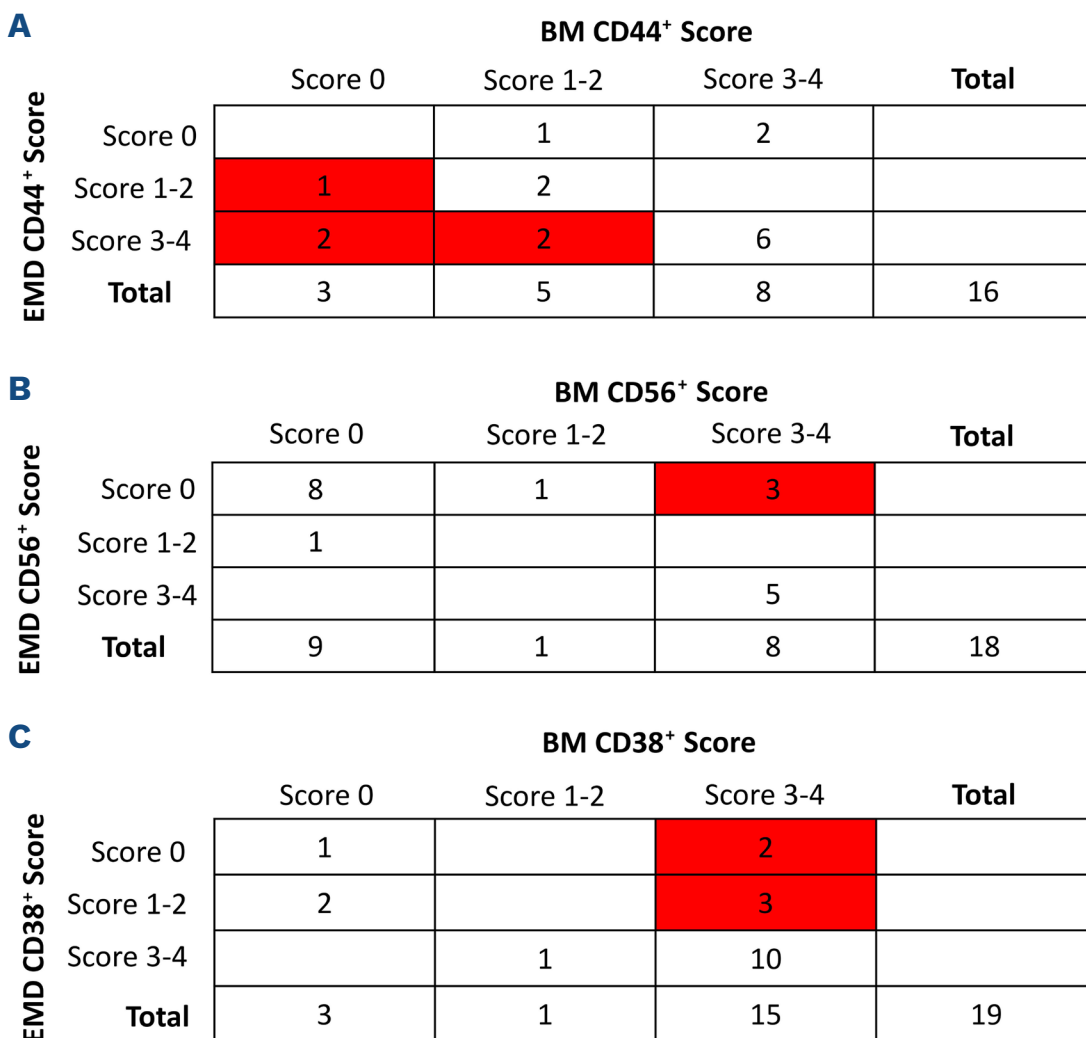


Figure 1. Immunohistochemistry score of matched bone marrow and extramedullary disease in multiple myeloma patients. Score 0 (rare CD38⁺ or CD44⁺ or CD56⁺ cells); score 1 (CD38⁺ or CD44⁺ or CD56⁺ cells, 5-24%); score 3 (CD38⁺ or CD56⁺ or CD44⁺ cells, 25-49%); score 4 (CD38⁺ or CD44⁺ or CD56⁺ cells >75%). (A) CD44 immunohistochemistry (IHC) score of matched bone marrow (BM) and extramedullary disease (EMD). (B) CD56 IHC score of matched BM and EMD. (C) CD38 IHC score of matched BM and EMD.

intent. Orchiectomy, a surgical procedure, was performed in two pts with an isolated EM relapse in the testes. Conventional chemotherapy was administered to three young (age ≤ 70 years) pts. Of the novel agents, proteasome inhibitors (PI) were the drugs most frequently used (40%), mainly in association with alkylating agents or anthracycline. Immunomodulatory drugs were administered mostly to pts already exposed to protease inhibitors (35%). Daratumumab was used in one pts in association with conventional chemotherapy. High-dose chemotherapy followed by autologous stem cell transplant (SCT) was performed in four of 20 (20%) pts, while allogenic SCT was performed in one pt.

The median OS from MM diagnosis in the entire study population was 49.3 months (95% confidence interval [CI]: 30.97-NA) while the median OS from EMD diagnosis was only 13.1 months (95% CI: 6.8-25.3). It confirms the poor outcome of this MM clinical feature as reported by

other studies.¹⁴

In conclusion, our data indicate that the lack of CD38 expression may occur in EMD lesions when compared to BM lesions with a discordant expression of CD44 and CD56. The lack or reduced expression of CD38 in a consistent percentage of EMD samples, may have a potential therapeutic impact involving the efficacy of anti-CD38 monoclonal antibodies in MM pts with EMD. As has already been reported in the literature,¹¹ pts with MM and EMD seem to have a lower response to anti-CD38 antibody than pts with MM without EMD. At the moment, however, this finding does not lead to a change of therapy for this subgroup of pts. Clinical data regarding the efficacy of daratumumab in EMD are needed, and clinical trial of daratumumab combined with bortezomib, cyclophosphamide, and dexamethasone in pts with EMD at diagnosis and first relapse is ongoing (EMN19 study, *clinicaltrials.gov*. Identifier: NCT 04166565).¹⁵

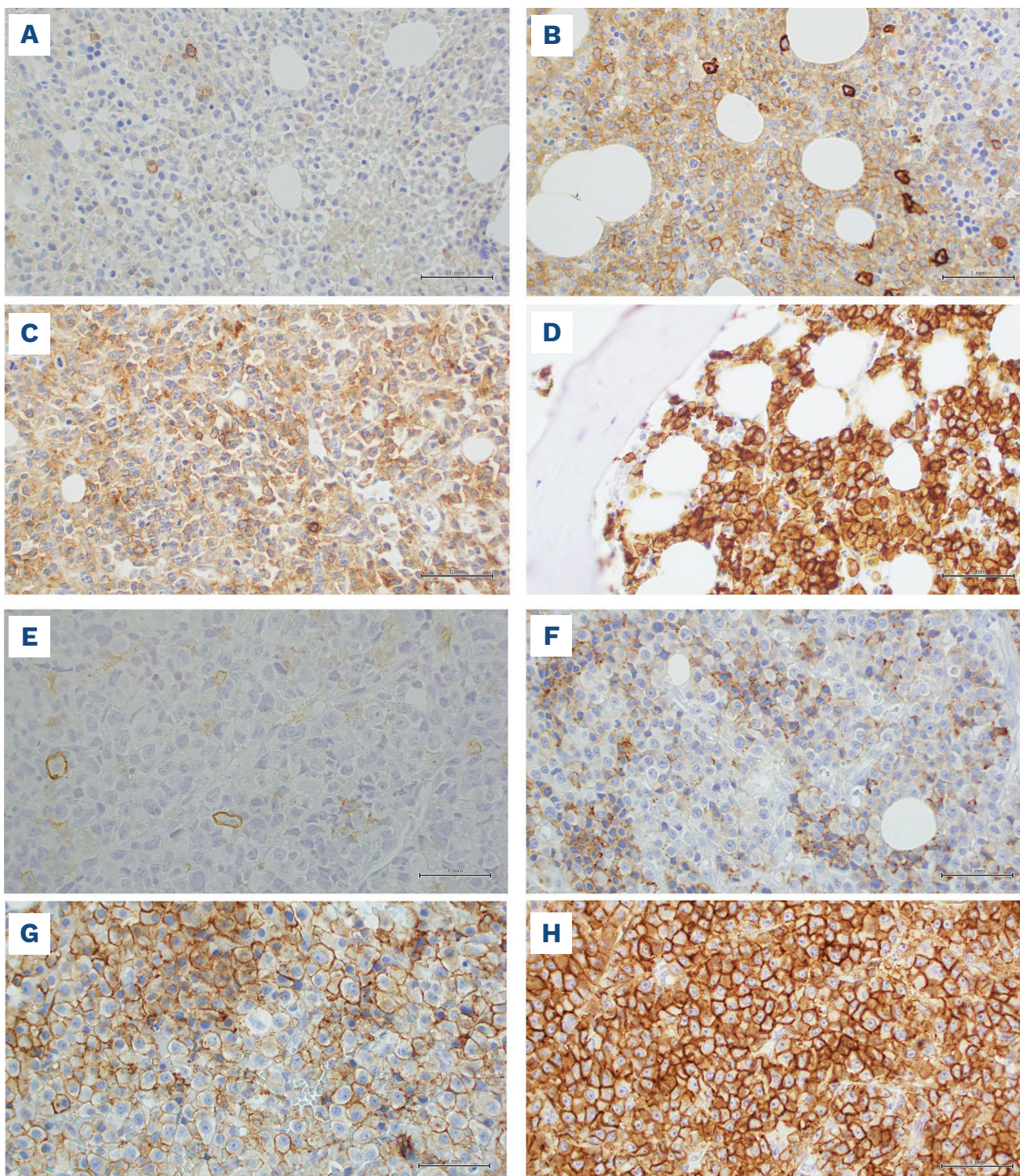


Figure 2. CD38 protein expression by plasma cells assessed by immunohistochemistry on bone marrow and on extramedullary sites. (A) Immunohistochemistry (IHC) score 0 in bone marrow (BM) (rare CD38⁺ cells, <5%). (B) IHC score 1 in BM (CD38⁺ cells, 5-24%). (C) IHC score 3 in BM (CD38⁺ cells, 25-49%). (D) IHC score 4 in BM (CD38⁺ cells >75%). (E) Soft tissue sample, IHC score 0 (rare CD38⁺ cells, <5%). (F) Soft tissue sample, IHC score 1 (CD38⁺ cells, 5-24%). (G) Liver sample, IHC score 3 (CD38⁺ cells, 25-49%). (H) Bowel sample, IHC score 4 (CD38⁺ cells >75%). Scale bars: 1 mm.

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Contributions

LN, FA and ABDP provided clinical data and enrolled patients. JBG, PS, NTI, VR and OL collected and processed the samples. RS, RV and SB performed the flow cytometry analysis. GT and GS performed the cytogenetic analysis. LN, FA and NG analyzed data and wrote the manuscript. FA performed statistical analysis. SR managed patients clinical data. LC read and provided comments. NG approved the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.