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ORIGINAL PAPER

Immunohistochemical Evaluation of 95 Bone Marrow Reactive Plasmacytoses

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Abstract We histologically and immunohistochemically studied 95 bone marrow (BM) reactive plasmacytoses. Ten biopsies from plasma cell myeloma (PCM) patients served as a control group. In addition, we studied 10 monoclonal gammopathy of undetermined significance (MGUS) cases. Histologically, plasmacytosis varied between 5% and 25% with an interstitial pattern of plasma cell (PC) distribution being characteristically displayed. Immunohistochemically, we did not find any CD56/NCAM nor cyclin D1 expression in all biopsies (95 of 95, 100%), not even a weak, doubtful one; PCs were all polyclonal and CD138 positive. On the contrary, myeloma-associated PCs showed monoclonality for κ- or λ- light chain and strong CD56/NCAM immunoreactivity (8 of 10, 80%); four of them were cyclin D1 positive. Osteoblasts exhibited similar CD56/NCAM expression in both groups. Our data confirm the diagnostic utility of CD56/NCAM in the phenotypic characterization of polyclonal plasma cells, suggesting an important role of this particular immunomarker in the BM trephine study of polyclonal versus neoplastic plasmacytic infiltrations.

Keywords CD56 · Cyclin D1 · Immunohistology · Bone marrow biopsies · Reactive plasmacytosis · Plasma cell myeloma

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Introduction

Reactive bone marrow (BM) plasmacytosis is observed with a variety of conditions, namely, chronic infections, autoimmune diseases, connective tissue and chronic granulomatous disorders, liver disease, diabetes mellitus, hypersensitivity states, drug-related agranulocytosis, iron deficiency, megaloblastic and haemolytic anaemia, haemopoietic and non-haemopoietic malignant disease, angioimmunoblastic lymphadenopathy and multicentric Castleman's disease [1–3]. In such reactive cases, plasma cells (PCs) usually account for 10–20%, but the level can rarely exceed up to 50% in BM trephine biopsy sections [3–5]; an interstitial pattern of plasma cell distribution, particularly adjacent to capillaries and macrophages, is characteristically displayed [1]. Moreover, reactive plasmacytosis (RP) may be associated with other reactive changes, including granulocytic hyperplasia, lymphoid follicles/aggregates, plasmacytic satellitosis (a central macrophage surrounded by plasma cells), lipid granulomas and increased numbers of macrophages, mast cells, eosinophils and megakaryocytes [1–2].

Owing to a potential overlap in the volume of plasma cell infiltrate, architectural pattern and cytomorphologic features, RP needs to be differentially diagnosed from immunosecretory disorders in general and specifically from plasma cell myeloma (PCM). In addition, a diagnosis of PCM in BM trephines depends on the percentage of monoclonal PCs detected by CD138 immunohistochemical staining (IHCS) in combination with the crucial detection of clonality by κ/λ IHCS; albeit, attempts have been made in order new immunohistochemical tests to be incorporated into the routine haematopathologic practice.

Among these new surrogate immunomarkers, CD56 (NCAM) is a credible one due to its frequent expression on neoplastic plasma cells. Particularly, CD56 is negative/

low in benign plasmacytoses and monoclonal gammopathy of undetermined significance (MGUS) cases, up-regulated in PCMs, and then down-regulated in synchrony with evolution to aggressive, plasmablastic or anaplastic myeloma or to extramedullary disease [6]. Of note, CD56 appears to have an important role in the specific biologic behaviour of PCM in individual patients regarding the presence of osteolytic lesions [6–8] and extramedullary dissemination [7–13]; indeed, $CD56^-$ PCM is a unique entity characterized by poor prognosis with high incidence of plasmablastic morphology, Bence Jones protein, renal insufficiency, thrombocytopenia, lack of osteolysis and extramedullary disease [6–8, 11, 14].

Since 1990 when van Camp et al. [15] first described CD56 expression on neoplastic PCs, only a few studies [6, 16–17] have, up to our knowledge, evaluated the use of CD56 antibody in the phenotypic differentiation of reactive PCs from myelomatous cells using immunohistochemistry. Although these displayed CD56 negativity in virtually all polyclonal RP cases examined (88/88; 4/5; 3/3), the immunohistochemical methodology applied so far, allowed for a "weak" immunoreaction to be noted in a limited number of non-neoplastic plasma cells. Inevitably, this finding would introduce evaluation problems, pertaining to subjectivity and interobsever variation, especially since a reproducible definition of "weak" immunostaining is not available at this point. Therefore, we investigated CD56 (NCAM) immunoexpression in a large series of well characterized polyclonal RPs and documented by using a computerized image analysis program.

Materials and Methods

Patients

A retrospective search of the registries of the Department of Pathology (Medical School, University of Thessaly) was performed to identify patients with polyclonal plasmacytoses. Clinical information was obtained from the patients' medical records and through contacting the primary physicians. Bone marrow samples from 95 patients with polyclonal plasmacytosis were identified. Any diagnostic consideration of PCM or MGUS was excluded by meticulous clinicopathologic correlation. The clinical problems that prompted the biopsies included 25 patients with chronic infections (leishmania, tuberculosis, brucellosis, sarcoidosis, toxoplasmosis, HIV, chronic hepatitis B and C), 20 patients with autoimmune diseases (rheumatoid arthritis, systemic lupus erythematosous), 10 patients with cirrhosis, 10 patients with diabetes melitus, 15 patients with neoplasia without marrow involvement and 15 patients with anaemia of variable aetiology (iron deficiency, megaloblastic anaemia, haemolytic anaemia). The blood smear showed plasmacytosis of different degree, whereas serum monoclonal immunoglobulin was not identified. Two years' follow up has not revealed neoplastic plasmacytic proliferation. Additionally, 10 biopsies from PCM patients and 10 from MGUS subjects were analysed.

Bone Marrow Biopsies

All biopsies were taken from the iliac crest, fixed in buffered formalin, decalcified in formic acid and routinely processed for paraffin embedding. Haematoxylin and Eosin, Giemsa stained slides and histochemical stains for iron (Perl's) and reticulin fibres (Reticulin) were studied in all cases.

Immunohistochemistry

CD56/NCAM Staining Immunohistochemical staining of paraffin sections for NCAM (CD56 clone 1B6; Novocastra, Newcastle, UK) was performed on an automated immunostainer using a streptavidin–biotin detection kit (Super-Sensitive MultiLink HRP detection kit /DAB, Biogenex). Antigen retrieval included placing the slide in citrate buffer, pH 6.0, in a pressure cooker for two minutes. Primary CD56 antibody was incubated for one hour at room temperature. Optimal primary antibody concentration was determined by serial dilutions, optimizing for maximal signal without background immunostaining. After extensive testing, a 1:100 dilution was deemed optimal under these conditions.

CD138, κ- and λ-Light Chains and Cyclin D1 Anti-syndecan-1 (CD138 clone M115; Dako, Glostrup, Denmark), κ- and λ-light chains (Dako) were included. Immunohistochemistry was performed using a heat-induced epitope retrieval technique and a universal secondary antibody kit that used a peroxidase conjugated labelled-dextran polymer (Dako EnVision+System, peroxidase DAB; Dako) on an automated immunostainer. A new rabbit monoclonal antibody for cyclin D1 (Neomarkers) was stained in all the cases using a similar protocol.

A grid ocular objective was used to count 500 cells over five high-power fields (×400) and the percentage of positive cells was reported as 0% to 100%, using 5% increments. The osteoblast layer was used as an internal positive control [6, 15].

Image Analysis

For image recording and analysis a semiautomated system was used. The hardware specifications were as follows: Intel Pentium IV, Matrox II Card Frame Grabber, Digital Camera Microwave Systems, and Microscope Olympus

BX-50. The software included: Windows XP Pro/Image Plus, version 3.0-Media Cybernetics (1997). Areas of interest, including immunostained cell subpopulations, were identified at a magnification of 40× and saved as high quality digital image files (JPEGs at resolution of 1,280×960) to complete the digital gallery. Image analysis process was based on an RGb 8-bit protocol with a range of 256 continuous values of intensity staining (0–255). Measurements of immunostained cell number per image were obtained by the implementation of a macro based on the DAB staining, which was used as chromogen substrate in the immunohistochemistry protocol. Light to dark brown colour range was considered to be acceptable for the identification of cell boundaries. Membranous ring like stain was observed to be complete in the majority of the examined cell subpopulations. In order to increase the level of segmentation and accuracy of the method, a diameter scale (8–30 μm) was used as a basis for the fragmentation of small clusters of accumulated cells. Using this utility, discrimination of indistinct cells was assessed. All measurements were performed inside an active window of 16,848 μm2.

Results

Plasmacytosis varied between 5% and 25%. An interstitial pattern of plasma cell distribution was demonstrated in all 95 BM trephine biopsies. There were also PCs around marrow capillaries, while any PC accumulations were not observed. PCs showed CD138 immunoexpression; stains with κ- and λ-light chains displayed polytypic results in all RPs. Moreover, plasma cells did not show any CD56 (NCAM) or cyclin D1 immunoreactivity; not even a weak, doubtful one. On the contrary, myelomatous PCs showed monoclonality for κ- or λ-light chains and CD56 immunoreactivity in 8 of 10 (80%) PCMs (Fig. 1). Four of them were cyclin D1 positive. The staining intensity was strong in all myeloma-associated PCs. Osteoblasts exhibited similar CD56 immunoreactivity in both groups (Fig. 2). With regard to MGUS cases, these were all, but one, negative for CD56; the 1 CD56+MGUS case contained a low-level (<2%) population of CD56+ PCs among 8% Igλ+PCs.

Discussion

RP needs to be differentially diagnosed from immunosecretory disorders, such as PCM, monoclonal gammopathy of undetermined significance (MGUS), primary amyloidosis and light chain deposition disease [1]. The latter two are the main categories of the monoclonal immunoglobulin deposition diseases (MIDD), which are plasma cell neoplasms and thus part of the spectrum of plasma cell myeloma; albeit, these closely-related entities produce an immunoglobulin molecule that accumulates in tissue prior to the development of a large tumour burden [4].

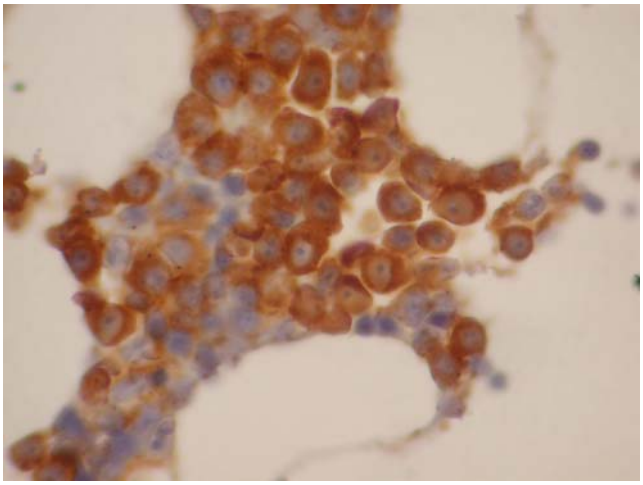

Fig. 1 Multiple myeloma case displaying strong CD56 immunoexpression

Although PCM is a straightforward diagnosis in most cases with significant plasma cell infiltration [18], it is sometimes not possible to be distinguished from reactive states of plasmacytosis on the basis of histology alone, as: (a) the volume of plasma cell infiltrate in MM can range from less than 5% to approximately 100% of the nucleated cells, while in most reactive cases being less than 20% and rarely exceeding up to 50%; (b) BM infiltration by PCM does not follow a recurrent pattern [18]; in fact, it can vary from randomly distributed clusters of PCs to larger foci, nodules or sheets, displacing normal marrow fat cells and haematopoietic elements, or complete obliteration of the marrow space [4, 19]. In RPs, the interstitial pattern of BM involvement is the typical one, with only a minority of cases showing plasma cell clustering [1]; in particular, PCs usually occur in small clusters of five or six (<10) cells around macrophages and arterioles [3–4]. Of note, perivascular plasma cell aggregates are not always benign and can be displayed in approximately one third of patients with myeloma [20]; (c) bi- or trinucleated forms; nuclear immaturity and pleomorphism; and, cytoplasmic/intranuclear inclusions can be present in reactive PCs [1, 4–5].

Immunohistochemistry, as an ancillary tool, can be of valuable help in the aforementioned differential diagnosis. By using antibodies to κ- and λ-light chains and determining the relative proportion of κ- or λ-reacting cells, the haematopathologist can either demonstrate immunoglobulin light chain restriction consistent with a monoclonal proliferation [4, 21] or a relatively balanced population of κ- and λ-containing cells, suggestive of a benign process [22]. Being consistent

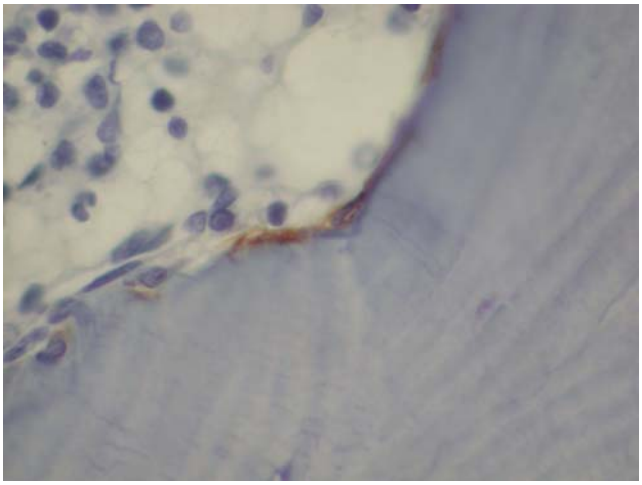

Fig. 2 Osteoblastic layer exhibiting CD56 immunoreactivity in a case of polyclonal plasmacytosis

with our data, immunohistology could similarly utilize CD56 as a tumor specific plasma cell marker in this particular distinction [3–4, 17]. In fact, CD56 is expressed in 70–90% of PCMs [16, 19, 23], whereas virtually all RPs, included within three previously published studies [6, 16–17], displayed negativity for CD56. However, Martin et al. [16] reported a single case of polyclonal plasmacytosis demonstrating CD56 positivity, while Ely and Knowels [6] regarded minor PC subpopulations with weak CD56 expression <10% as negative, suggesting the need of setting cut off points. Such discrepancies may be attributable in part to different antibodies/immunohistochemical procedures (CD56 clone 1B6 Novocastra, CD56/NCAm Synbio/Monosan, CD56/NCAM Novocastra, Leu-19Beckton Dickinson) or may suggest low-level clonal PC populations [17].

With regard to the non-secretory PCM, a rare (3%) clinical variant with no evidence of immunoglobulin production in the serum or urine, the diagnosis is usually established by immunohistological detection of PC aggregates, atypia and cytoplasmic light chain restriction [3]. In this variant, the role of CD56 immunoexpression can be fundamentally dual as: (a) it can offer great diagnostic aid especially in these cases characterized not only by failure to secrete but to synthesize Ig molecules as well [4]; cytoplasmic Ig staining is subsequently of no use and (b) it can be utilized as a reliable discriminator between this variant and striking RPs observed in the context of non malignant conditions such as liver disease, chronic granulomatous disorders, connective tissue disorders, hypersensitivity states and drug-related agranulocytosis [2].

Another possible application of CD56 could be theoretically expected in those MGUS cases without a demonstrable monotypic restriction, either of a minimal plasma cell burden or a small clone present in a background of reactive plasma cells [3–4]. In this regard, it has been suggested, based on flow cytometric studies, to use a combination of anti-CD56 and anti-CD19 antibodies, given that two immunophenotypically different PC subpopulations can be distinguished in MGUS patients; one with a normal/polyclonal (CD19+/CD56−) and the other with an aberrant/monoclonal (CD19−/CD56+, CD19−/CD56− or CD19+/CD56+) phenotype [4, 24]. In the present study, there was only a single MGUS case (1/10), which displayed CD56 immunoreactivity in a minority of the monotypic PCs. The significance of our finding, if any, is unclear. Overall, the pertinent literature seems to indicate that CD56 immunostaining could trace an incipient myeloma clone in a background of MGUS plasmacytosis, but the practical application is limited by the small number of the stained cases, the small number of the CD56+PCs, and the limited sensitivity of the technique.

Moreover, by documenting the immunophenotype of myeloma-associated cells, along with a panel of antibodies, CD56 immunomarker should be of great value to: (a) the diagnosis of PCM in small biopsies or when plasma cell clonality is difficult to assess because of background staining; (b) the assessment of minimal residual disease (PCM) following therapy [3, 19, 25]; (c) the follow-up of MGUS patients in anticipation of PCM transformation [17, 23]; approximately 25%-30% MGUS cases eventually develop a plasma cell dyscrasia or related process after an interval to occurrence, ranging up to 29 years, at a rate of 0.8% each year [3–4, 22]; (d) the distinction of MGUS from initial or residual PCM [3, 18, 24]; and (e) the discrimination of PCM from non-Hodgkin's lymphomas (NHLs) with plasmacytoid differentiation; apart from the rarely encountered microvillous lymphomas [6].

In conclusion, our data confirm the diagnostic utility of CD56 in the phenotypic characterization of reactive PCs, suggesting a crucial role for this particular immunomarker in: (a) the bone marrow trephine study of extensive polyclonal versus neoplastic plasmacytic infiltrations; and (b) the diagnosis of PCM in small biopsies, when plasma cell clonality is difficult to assess because of background staining or in these non-secretory PCM cases, characterized not only by failure to secrete but to synthesize Ig molecules as well. With regard to its utility in: (a) the diagnosis of those MGUS cases without a demonstrable monotypic restriction, either of a minimal plasma cell burden or a small clone present in a background of reactive PCs; and (b) the follow-up of MGUS subjects in anticipation of PCM transformation, this should be further investigated in a large series of MGUS cases.

Conflict of interest statement We, all authors, declare no conflict of interest. It is our will so as both figures (illustrations) to be reproduced in black and white.

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