

CD23 expression in plasma cell myeloma is specific for abnormalities of chromosome 11, and is associated with primary plasma cell leukaemia in this cytogenetic sub-group

CD23 is a low-affinity Fc receptor for IgE and is pivotal in IgE homeostasis (Hibbert *et al*, 2005). CD23 exists in two isoforms; CD23a, present on mature B-cells, and CD23b, which requires induction by interleukin-4 for activation on T cells, monocytes, Langerhans cells, eosinophils, and macrophages. CD23 is also constitutively expressed on Epstein-Barr virus (EBV) transformed B-lymphoblasts, and is therefore also known as EBV surface antigen. The *FCER2* gene on chromosome 19p13.3 encodes the CD23 45 kDa protein containing 321 amino acids. A multifunctional protein, CD23 has activities associated with cytokine modulation and B-cell growth factor function.

The diagnosis of plasma cell myeloma (PCM) relies on the presence of an M-protein in the serum/urine, a clonal proliferation of plasma cells, and related organ/tissue damage, the latter defined as anaemia, hypercalcaemia, lytic bone lesions, renal insufficiency, hyperviscosity, amyloidosis, or recurrent infections (McKenna *et al*, 2008). Chromosomal abnormalities are identified in >90% of cases, often involving *IGH@* on chromosome 14q32, as well as chromosome bands 11q13, 4p16.3, 16q23, 6p21, and 20q11 (Avet-Loiseau *et al*, 2007). Numerical and structural chromosomal irregularities are also frequent, the most common being 1q gains and 13q14 deletions. PCM usually shows monotypic expression of intracytoplasmic light chain, expression of CD79a, strong CD38, CD138, and VS38c. A majority of cases express CD56, but the cells usually lack CD19 and CD20. In contrast, normal plasma cells are mainly CD19⁺ and CD56⁻ (Lin *et al*, 2004). Expression of cyclin D1 and CD20, and lack of CD56 has previously been reported in association with the t(11;14)(q13;q32) (Hundemer *et al*, 2007). Primary plasma cell leukaemia (PCL) is immunophenotypically similar, with the exception being common negativity for CD56 (Garcia-Sanz *et al*, 1999). Although CD23 expression is well characterized in other haematological malignancies, to our knowledge evaluation of CD23 in plasma cell myeloma has not previously been reported.

We examined 50 diagnostic PCM bone marrows (including three cases of primary PCL) for CD23 expression by immunohistochemistry. Five cases were also evaluated for CD23 expression by flow cytometry. Vector[®] Laboratories CD23 mouse monoclonal antibody with Vector[®] Antigen Unmasking Solution and high temperature antigen unmasking protocol was used for detection on paraffin sections from decalcified

bone marrow biopsies when available and clot sections when no core biopsy was available. Immunophenotypes from each case were derived from routine flow cytometry performed at diagnosis using previously reported methods (Harrington *et al*, 2009). Assessment of CD19, CD20, CD45, CD56 and intracytoplasmic immunoglobulin light chain expression was done in most cases (49 out of 50). CD23 expression was correlated with presenting laboratory data, including: blood counts (white blood cell count, red blood cell count, Haemoglobin, mean cell volume, mean cell haemoglobin concentration, red cell distribution width, and platelet count), M-protein level, creatinine, beta-2 microglobulin, serum calcium, and cytogenetics. Cytogenetic analysis, including unstimulated culture, chromosome analysis and fluorescent *in situ* hybridization (FISH) of marrow cells, was performed using previously reported methods (Heerema *et al*, 2007). Commercially available DNA probes that detect characteristic clonal rearrangements in multiple myeloma [deletion p53, deletion 13q14, t(11;14), t(4;14) and t(14;16)] were obtained from Vysis (Des Plaines, IL) and used per the manufacturer's instructions. For most cytogenetic analyses 20 metaphases were examined; for FISH, 200 interphase cells were analyzed for each probe in each case. Where t(11;14)(q13;q32) cytogenetics were identified, cyclin-D1 immunohistochemistry was also performed using the Thermo Scientific rabbit IgG protocol targeting the C-terminus of human cyclin-D1. Statistical analysis was performed using the Statistical Package for the Social Sciences software for Macintosh, version 11.0 (SPSS, Inc., Chicago, IL, USA). The study protocol was approved by the Medical College of Wisconsin internal review board.

Of 50 PCM cases examined for CD23 expression, five (10%) showed uniform strong membranous positivity (Fig 1); three also showed golgi staining. CD23 expression was confirmed by flow cytometry in two of five cases. All five CD23 positive cases displayed abnormalities of chromosome 11 by conventional cytogenetics and/or FISH. Four of these cases contained a t(11;14)(q13;q32), while the fifth case showed a trisomy 11 by conventional cytogenetics. In total, t(11;14)(q13;q32) was identified in 10/50 PCM cases (20%). All t(11;14)(q13;q32) cases were positive for cyclin-D1 by immunohistochemistry (Bergsagel *et al*, 2005). No significant differences in presenting laboratory data or other immunophenotypic features were identified between the t(11;14)(q13;q32) cases that were CD23 positive and negative. Associations of immunophenotypic

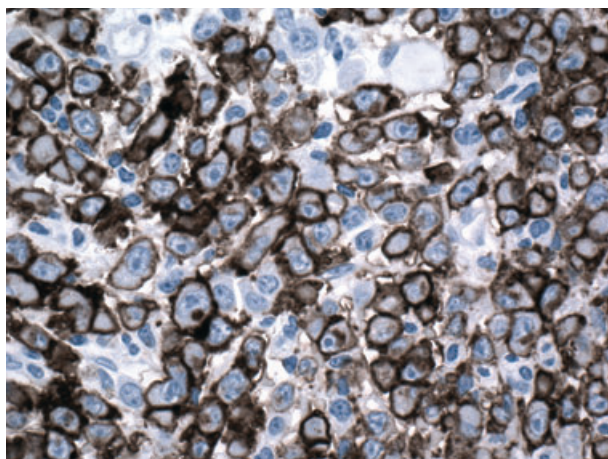


Fig 1. CD23 showing strong membranous and focal Golgi staining in malignant plasma cells. Original magnification $\times 1000$.

Table I. Summary of immunophenotypic associations with CD23, t(11;14) and plasma cell leukaemia/myeloma.

	CD23 ⁺	CD23 ⁻	P value
CD19 ⁺	2/5	0/41	0.010
CD56 ⁺	1/5	33/43	0.021
CD45 ⁺	3/5	16/38	0.20
	t(11;14) Positive	t(11;14) Negative	
CD20 ⁺	4/10	2/35	0.016
CD56 ⁺	4/10	29/36	0.020
CD45 ⁺	3/10	16/34	0.46
CD23 ⁺	4/10	1/36	0.005
	Plasma cell leukaemia	Plasma cell myeloma	
CD19 ⁺	2/4	0/42	0.006
CD23 ⁺	3/4	2/46	0.002
CD45 ⁺	4/4	16/39	0.039
CD56 ⁺	1/4	33/44	0.069

features with CD23 expression, t(11;14)(q13;q32), and leukemic presentation are provided in Table I. Compared to negative cases, CD23-positive PCM was more likely to express CD19 and less likely to express CD56. Cases with t(11;14)(q13;q32) also more frequently expressed CD20 and less frequently expressed CD56. Three of four cases of primary PCL were CD23 positive. PCL cases were more likely to express CD45 and CD19 and less likely to express CD56 than non-leukemic PCM.

In summary, we found strong, uniform expression of CD23 in 10% of PCM cases. This study is the first to our knowledge to examine the expression of CD23 in PCM. This expression did not correlate with presenting laboratory data, although the number of cases was small. Expression of CD23 was, however, associated with abnormalities of chromosome 11, specifically

t(11;14)(q13;q32) in four of five cases, and trisomy 11 in the fifth. Previous reports have described a high prevalence of t(11;14)(q13;q32) in PCL (Avett-Loiseau *et al*, 2007). Although our study only included four cases of PCL, 4/4 had a t(11;14)(q13;q32) and three expressed CD23. Our study confirms the association of lack of CD56 in primary PCL, and also revealed an association of CD19 and CD45 expression with this subgroup of PCM, which are previously unreported findings. Finally, our study corroborates reported associations of t(11;14)(q13;q32) with expression of CD20 and lack of CD56 in PCM (Hundemer *et al*, 2007).

Although CD23 was strongly associated with the t(11;14)(q13;q32), only 40% of cases with this translocation were CD23⁺. We found no significant association between presenting features within this cytogenetic cohort based on CD23 expression. However, examination of a larger set of cases is necessary to assess the clinical relevance of CD23 expression in PCM carrying the t(11;14)(q13;q32). Although the role of CD23 in PCM is unknown, its expression in a minority of cases could represent a therapeutic target in the subset of positive cases (Pathan *et al*, 2008).

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The difficult distinction between antiphospholipid syndrome and thrombotic thrombocytopenic purpura

The unusual case of thrombotic thrombocytopenic purpura (TTP) in association with antiphospholipid syndrome (APS) reported by Díaz-Cremades *et al* (2009) present several important considerations in the management of both these disorders. Firstly, the clinical presentation reported emphasises the need for 'two-hits' for the development of clinical features in APS, especially the thrombotic episodes (Levine *et al*, 2002). The two-hit theory of APS explains the common observation that antiphospholipid antibodies are persistently present in the circulation of these patients but the thrombotic events occur only occasionally. Very often a recent or concomitant infectious process or an inflammatory or traumatic stimulus forms the 'second hit' to initiate the thrombosis. The present patient had no prior history of thromboses or miscarriages when APS was suspected, but presented with a right-sided stroke in a short period of time, when it was tested and confirmed. The report does not however explain any triggers for the onset of hemiparesis although subclinical infection or inflammatory states leading to vascular injury may have led to the acute episode. It is likely that such a 'subclinical event', which is likely to be an inflammatory stimulus (as evidenced by the positive autoantibodies) might have contributed to the decreasing platelet count and haemoglobin concentration (without a diagnosis of TTP) already noted at this point in the patient. It is also useful to note in this context that TTP itself is a two-hit event, as evidenced by the ADAMTS13 knockout mice model, where ADAMTS13 deficiency itself does not cause thrombosis.

Secondly, the report demonstrates the difficulties in diagnosing TTP in complex presentations. TTP has been associated with other clinical conditions including autoimmune diseases, such as systemic lupus erythematosus (SLE), wherein a strong suspicion is necessary especially as many of these conditions can also cause low platelet counts with anaemia due to other reasons (Porta *et al*, 2005). In the present patient, one of the characteristic microscopic features of TTP, schistocytes, only appeared 2 days after the development of severe thrombocytopenia, and haemolytic anaemia stressing the need for persistent observance for this finding. This has been stressed by the British Committee for Standards in Haematology guidelines, which suggests a delay of up to 48 h, can occur before the appearance of schistocytes on the blood film.

Thirdly, the distinction between APS and TTP presented in the discussion as a controversial subject is worth re-examining. Although the 'TTP' in the report responded well to plasma exchange and rituximab treatment, it is not clear whether the antiphospholipid antibodies also disappeared at the same time as the antibodies to ADAMTS13. If latter is the case, it would suggest that the pathophysiological reasons for the development of recurrent brain ischaemia are likely to be related to APS rather than *purely* TTP. Also, the thrombocytopenia and haemolytic anaemia attributed to TTP could have been antibody-related autoimmune cytopenias. Here, it is useful to note that plasma exchange has been shown to improve thrombocytopenia related to APS in a previous case report (Satomi *et al*, 1998). Plasma exchange is also a recommended