ABSTRACT

Aim Previous studies showed that CD200 expression is a prognostic factor for plasma cell myeloma (PCM), but the prognostic effect is conflicting between studies. We studied CD200 protein expression and the stability of expression in PCM to clarify its potential utility in diagnosis, prognosis and monitoring of disease.

Method CD200 expression was studied in 77 cases of PCM by immunohistochemistry on paraffin sections from decalified bone marrow biopsies.

Result There were 16 newly diagnosed cases and 61 post-treatment cases from 54 patients: 37 men and 17 women, with a median age of 62 years (range, 41–88 years). CD200 demonstrated moderate to strong membrane expression in positive cases. Fifty-six of 77 cases (73%) showed CD200 expression. Twenty of the 22 (91%) patients with serial specimens demonstrated stable CD200 expression (n=15) or lack of CD200 expression (n=5). One patient lost CD200 expression, while another one gained CD200 expression during treatment. The clinical, pathologic and cytogenetic features between the CD200+ group and the CD200− group were similar in most instances. However, CD200 expression was associated with a shorter event-free survival (EFS) on the contrary, another study suggested that PCM patients with the loss of CD200 expression had a more clinically aggressive disease. To further clarify the prognostic significance of CD200 expression in PCM and to evaluate the stability of CD200 expression during the course of treatment, we studied CD200 expression in 77 cases of PCM from 54 patients, including 22 patients with 2–3 serial biopsy specimens.

Conclusions CD200 is expressed in a majority of PCM cases, and the expression is stable during the treatment process. Therefore, immunohistochemical expression of CD200 is a useful marker for the diagnosis and follow-up of PCM.

INTRODUCTION

Plasma cell myeloma (PCM) is a bone marrow-based malignant plasma cell neoplasm. It comprises about 10–15% of haematologic neoplasms, and causes 20% of deaths from haematologic malignancies. Despite extensive clinical research in PCM in the past several decades, it remains an incurable malignancy. The diagnosis of PCM is largely based on demonstration of clonality, and an aberrant immunophenotype of the malignant plasma cells characterised by several well-known markers including CD38, CD138, CD19, CD20, CD45 and CD56. In recent years, microarray or genome sequencing studies have identified new markers related to PCM including CD200.

CD200 (OX-2 antigen) is a type I immunoglobulin superfamily membrane glycoprotein. It is expressed in multiple cell types, including B cells, a subset of T cells, dendritic cells, endothelial cells, and in the peripheral and central nervous systems. CD200 interacts with CD200R, an immunoglobulin superfamily inhibitory receptor expressed primarily on myeloid/monocyte lineage cells and subset of T cells, and has a suppressive effect on monocyte and T cell-mediated immune response. CD200 is expressed by some non-Hodgkin B-cell lymphomas, including chronic lymphocytic leukaemia (CLL)/small lymphocytic lymphoma, hairy cell leukaemia, and angioimmunoblastic T cell lymphoma, but is absent in mantle cell lymphoma (MCL). It is also expressed by classical Hodgkin lymphoma but not nodular lymphocyte-predominant Hodgkin lymphoma. Additionally, CD200 has been shown to be expressed in acute myeloid leukaemia and B lymphoblastic leukaemia, in a number of carcinomas, and in malignant melanoma.

Recent studies demonstrated that CD200 is expressed by malignant plasma cells in PCM but not by benign plasma cells, and may serve as a prognostic marker. Two studies demonstrated that CD200 expression was associated with a shorter event-free survival (EFS). On the contrary, another study suggested that PCM patients lacking CD200 expression appeared to have a more clinically aggressive disease. To further clarify the prognostic significance of CD200 expression in PCM and to evaluate the stability of CD200 expression during the course of treatment, we studied CD200 expression in 77 cases of PCM from 54 patients, including 22 patients with 2–3 serial biopsy specimens.

MATERIAL AND METHODS

Case selection
A total of 77 cases of PCM diagnosed between 2003 and 2012 were retrieved from the Department of Pathology, Microbiology, and Immunology at Vanderbilt University Medical Center database with approval from the Institutional Review Board at Vanderbilt University Medical Center. These cases are from 54 patients, with 22 patients having 2–3 serial biopsies. All cases have a plasma cell load >20% by CD138 immunohistochemistry. Corresponding medical records were reviewed to obtain clinical information, including laboratory data, treatment regimens, response to therapy, progression-free survival (PFS) and overall survival (OS). Morphologic, immunophenotypic, and cytogenetic data were reviewed to confirm the diagnosis according to the 2008 WHO criteria.

Immunohistochemistry
CD200 immunohistochemical staining was performed on B5-fixed paraffin-embedded bone marrow biopsy specimens from the above 77 cases on an automated immunostainer (Leica Bond-Max...
IHC stainer, San Diego, California, USA). The 4 μm-thick tissue sections were deparaffinised and underwent heat-induced antigen retrieval using the Bond Max Epitope Retrieval 2 solution for 20 min. The sections were incubated with a goat anti-human CD200 antibody (R&D Systems, Minneapolis, Minnesota, USA) for 1 h at a 1:150 dilution. The Bond Refine Polymer detection system was used for visualisation.

Three cases of CLL with significant bone marrow involvement were used as positive controls, and three cases of MCL extensively involving the bone marrow served as negative controls. A second group of negative control consisted of four bone marrow biopsy cases with reactive polyclonal plasmacytosis. All immunostained slides were evaluated by three pathologists and were scored as either positive or negative by using moderate to strong membranous staining in 25% of plasma cells as a cut-off.

Statistical analysis
Patient survival was analysed using the Kaplan–Meier method and compared using the log rank test. The Fisher exact test was used to compare differences between the CD200+ and CD200− groups. A p value of less than 0.05 was considered statistically significant.

RESULTS
The study cohort included 37 men and 17 women, with a median age of 62 years (range, 41–88 years). Of the 77 cases from these 54 patients, 16 were newly diagnosed and 61 were post-treatment cases, including chemotherapy alone and chemotherapy plus stem cell transplant (SCT).

CD200 demonstrated moderate to strong membrane expression in positive cases. It was strongly and diffusely positive in all three cases of CLL, but negative in all three cases of MCL. The reactive plasma cells in four benign marrow biopsies were all negative for CD200 expression, similar to previous results from the literature. Fifty-six of 77 cases (73%) or 38 of 54 patients (70%) showed CD200 expression in myeloma cells (figure 1). Of the 16 newly diagnosed cases, 14 were CD200+ (87.5%). Twenty-two patients had serial biopsies with intervals between biopsies ranging from 3 months to 2 years. Twenty of the 22 (91%) patients with serial specimens demonstrated stable
expression (n=15) or stable lack of expression (n=5) of CD200, including prechemotherapy versus postchemotherapy or pre-SCT versus post-SCT pairs. One patient lost CD200 expression while another one gained CD200 expression during treatment, and both patients died a few months after the change in expression was detected.

The clinical, pathologic and cytogenetic features between the CD200+ group and the CD200− group were similar in most instances (table 1), including patient age, gender, light chain restriction, lactate dehydrogenase (LDH), and haemoglobin. Importantly, an abnormal karyotype was seen at a similar frequency in CD200+ cases and CD200− cases (43% vs 43%, p=1.0). Similarly, the frequency of t(11;14), t(4;14), del(13q), and del(17p) detected by fluorescent in situ hybridization (FISH) were all similar between the CD200+ and CD200− groups (p>0.05). However, CD200 expression was associated with a lower serum β2-microglobulin level (≤3.5 mg/dL in 50% of CD200+ group vs 14% of the CD200− group, p=0.03).

All patients received standard treatment regimens including bortezomib, lenalidomide and dexamethasone with slight variability. Thirty-four of the 54 patients (63%) also received SCT, including 32 patients receiving autologous SCT and two receiving allogeneic SCT. There was no statistically significant difference in the proportion of cases treated with SCT between the CD200+ and CD200− groups (p=0.37). There was also no significant difference in median OS or PFS between the CD200+ and CD200− patients, both when all cases were considered, and when only the newly diagnosed cases were considered (figure 2).

**DISCUSSION**

We studied the CD200 expression in 77 cases of PCM from 54 patients. Similar to previous studies, we identified that CD200 is expressed by 73% of PCM cases but not by reactive polyclonal plasma cells. However, CD200 expression is not associated with patient survival in our cohort. Additionally, 22 patients from our study who had serial biopsies, demonstrated stable CD200 expression during the course of treatment.

Moreaux et al studied 112 newly diagnosed PCM patients using gene expression profiling and flow cytometry in a subset of cases, and found that 78% of myelomas express CD200. They determined that CD200 expression is associated with high frequency of lambda light-chain expression and worse EFS, while absence of CD200 on plasma cells correlates with age ≥65 years. A similar study by Olteanu et al included 76 patients (44 newly diagnosed and 32 previously treated myeloma patients) using flow cytometry and immunohistochemistry confirmed the worse EFS in CD200+ myeloma patients. They further found CD200 expression was associated with low LDH and low haemoglobin level. By contrast, another flow cytometry study included 32 cases of PCM, mostly post-treatment cases, and found CD200 negative myeloma was significantly prevalent in ‘PR’ subtype of myeloma, a subtype defined by gene expression signature, and associated with poor clinical outcome. In other words, CD200 positive myeloma appeared to have better outcome than CD200 negative cases.

In our cohort, CD200 was not expressed by reactive plasma cells but expressed by a large subset of myeloma cases (73%), similar to previous studies demonstrated by immunohistochemistry and flow cytometry. By contrast with these studies, however, CD200 expression was not associated with a change in OS and PFS in our cohort, either when all patients were included, or when only newly diagnosed patients were analysed. However, there were a limited number of new diagnosis cases (16/77 cases). This was further confirmed by our cytogenetic correlation. As shown in table 1, an abnormal karyotype is seen at a similar frequency in CD200+ cases and CD200− cases (43% vs 43%, p=1.0). Specifically, the prevalence of deletion 17p, a cytogenetic abnormality associated with poor outcome in myeloma, was not significantly different in CD200+ cases (24%) compared to CD200− cases (14%) (p=0.70). Similarly, the positive rate of t(11;14), a cytogenetic abnormality associated with better outcome in myeloma, was not significantly different in CD200+ cases vs CD200− cases either (24% vs 43%) (p=0.29). Additionally, we did not observe any difference in age, light-chain usage, and haemoglobin levels between CD200+ and CD200− groups. Instead, lower serum 2-microglobulin levels were statistically more prevalent in CD200+ cases than in CD200− cases (p=0.03) in our cohort, which was not observed in previous studies.

Previous studies demonstrated that CD200-CD200R interaction has a suppressive effect on monocyte and T cell-mediated immune response. This could explain the worse EFS of CD200+ myelomas demonstrated by some studies. However, it does not fit into the negative impact of lack of CD200 expression as shown by another study and the lack of impact on survival in our analysis. This suggests that the function of CD200 and its regulation in PCM is complex and further study is required.

It is obvious that CD200 is a useful diagnostic marker for PCM. To serve as a marker for follow-up or minimal residual disease detection, its expression must be specific and stable during disease evolution and treatment process. CD200 is expressed in most cases of PCM but not in reactive plasma cells, as has been demonstrated by us and others using both immunohistochemistry and flow cytometry, suggesting that its expression is relatively specific for malignant plasma cells.
However, information about CD200 expression stability is sparse in the literature, especially using immunohistochemistry. We studied 22 myeloma patients with serial marrow biopsies, either before and after chemotherapy or before or after SCT with recurrence, and found 91% of patients demonstrate stable CD200 status with 15 of 16 (94%) previously positive cases still positive after chemotherapy and/or SCT. This finding is similar to previous results demonstrated by flow cytometry. To our knowledge, this is the first study using immunohistochemistry that demonstrated the stability of CD200 expression in myeloma cells. Although CD200 protein expression detected by flow cytometry and by immunohistochemistry showed a good concordance in a previous study, plasma cells are significantly underestimated by flow cytometry. Therefore, the use of CD200 immunohistochemistry for monitoring PCM, especially for the detection of minimal residual disease has a significant advantage over flow cytometry. The use of this marker for minimal residual disease detection in combination with other markers, is further supported by its stable expression in myeloma cells as suggested previously. Two of the 22 patients were exceptional: one patient lost CD200 expression while another one gained CD200 expression after chemotherapy and SCT. Both patients died a few months after the change in CD200 expression was detected. Therefore, the significance regarding the loss or gain of CD200 expression is unclear.

In summary, our results demonstrated no significant difference in median OS and PFS between CD200+ and CD200− myeloma patients, highlighting the conflicting nature of the literature on CD200 expression in myeloma prognosis. However, we present the first evidence by immunohistochemistry that CD200 is stably expressed in myeloma cells throughout the disease course. Although it is not a prognostic marker, CD200 can serve as a reliable immunohistochemical marker for myeloma diagnosis, follow-up and therapy monitoring.

Figure 2 Overall survival (OS) and progression-free survival (PFS) in all CD200+ vs CD200− plasma cell myeloma patients (A and B, n=38 for CD200+ vs n=14 for CD200−) or newly diagnosed patients (C and D, n=14 for CD200+ vs n=2 for CD200−).

Take home messages

- CD200 is a reliable immunohistochemical marker for plasma cell myeloma (PCM) diagnosis and disease follow-up: it is expressed in a majority of PCM cases, and the expression is stable during the treatment process.
- CD200 expression does not correlate with clinical outcome.

Contributors JJD, DJL and ASK contributed to the planning, conducting and reporting of the research and writing of the manuscript. SL contributed to the planning, conducting and reporting of the research and writing of the manuscript, and also serves as guarantor responsible for the overall content.

Competing interests None.

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