Haematologists usually over-estimate the percentage of CD138+ plasma cells in marrow biopsies

Sir,
Quantification of plasma cells on CD138 immunostained bone marrow core biopsies yields consistently higher results than corresponding aspirate samples, leading to the conclusion that aspirate samples underestimate the true plasma cell burden.[1−7] Since 2014, the International Myeloma Working Group (IMWG) recommends that both aspirate and core biopsy be used for quantification of plasma cells, and in cases of disparity, the highest value should be used.[8] However, the diagnostic criteria do not specify the method of plasma cell enumeration.

Given the clinical importance of accurate plasma cell quantification, we conducted a nationwide survey to assess the variability of plasma cell enumeration by New Zealand haematologists on immunohistochemically stained core biopsies. Six bone marrow core biopsies showing a range of plasma cells were selected and 12 images of representative regions with >250 nucleated cells were taken. Eight images were immunostained for CD138 and four for MUM1; six images were magnified at 100× and the other six at 40× (four CD138 and two MUM1 for each magnification). The proportion of plasma cells was determined by manually counting all cells on each image. The images were then sent as a survey to practising haematologists in New Zealand and respondents were asked to quantify the percentage of plasma cells in each image.

There were 20 responses comprising 238 individual estimates, of which 85% (203) were an over-estimate compared to the counted plasma cell percentage. The average absolute difference for percentage of plasma cells was 14.2% higher in the estimates compared to the true plasma cell percentage. The second key finding was that MUM1 stained images were associated with approximately half the level of over-estimation compared to CD138 stained images (mean over-estimate 7.1% vs 17.7%) (Fig. 1).

Our data likely reflect visual illusions associated with visual estimates. Perceptual psychologists have concluded when observers visually estimate the numbers of objects too numerous to count (numerousity estimates), they tend to over-estimate numbers if the objects are dispersed uniformly against the background.[9,10] Eighty-three percent of myeloma patients have an interstitial, micro-aggregate or diffuse pattern of myeloma cell distribution on core biopsies, which are evenly dispersed, leading to an over-estimation bias.[8] This perceptual bias is exaggerated further when using the CD138 immunostain, as cytoplasmic/membrane staining makes plasma cells appear larger, whereas MUM1 uptake is confined to the plasma cell nucleus, leading to less over-estimation.

Our results have been replicated in two prior studies which used objective methods of plasma cell enumeration by computerised assisted image analysis. Both papers showed that haematologists and specialised lab technicians over-estimated the percentage of plasma cells on CD138 stained core biopsies, with a larger degree of over-estimation at higher levels of plasma cell infiltrate.[4,5]
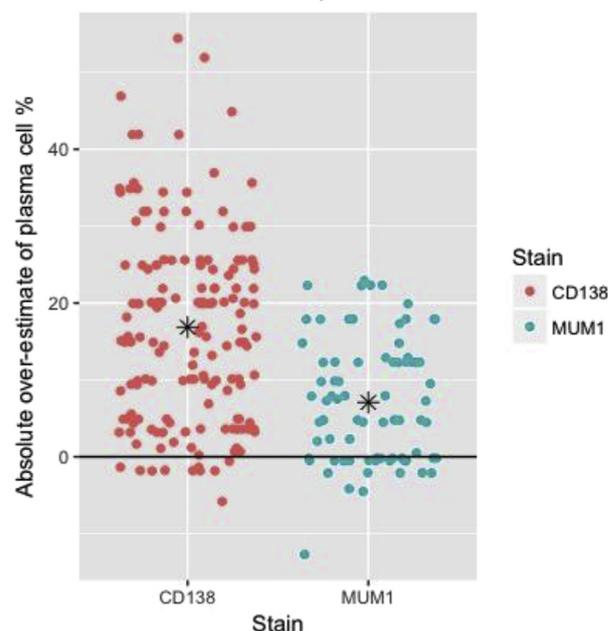

Fig. 1 Jitter plot showing the absolute over-estimate of plasma cell percentage using CD138 (salmon dots) vs MUM1 (green dots) immunostain. The black asterisk represents the median over-estimate for each immunostain.

This over-estimation, and hence over-diagnosis, might lead to unnecessary treatments with profound physical and psychological impact on the patients and their families. Three recent studies have shown that 67%, 95% and 57% of patients who were previously labelled as monoclonal gammopathy of undetermined significance (MGUS) on aspirate samples (bone marrow plasma cell <10%), were found to have ≥10% plasma cells on CD138 immunostained core biopsy.[1−3] Of those 'upgraded' patients, a corresponding 5%, 39.7% and 4.2% had ≥60% plasma cells on core biopsy, reclassifying them as having a myeloma defining event, with consequential treatment.[1−3] Our data suggest that some of these patients would have been wrongly 'upgraded' due to visual over-estimation, leading to unnecessary treatment and potential toxicity.

Limitations of this study include small sample size and the use of photographic images. Furthermore, it is noted that the magnitude of over-estimation (mean of 14.1%) is smaller than the degree of under-estimation when using aspirate samples compared to immunostained core biopsy samples. Four reports have shown the degree of under-estimation on aspirate samples compared to core biopsy samples ranges between 17.1% and 28% (mean 20.5%).[2−4,6] This difference suggests that whilst CD138 immunostained core biopsies leads to systemic over-estimation, results obtained from aspirate samples are truly an under-estimate, due to inherent technical issues including dilution by sinusoidal blood and failure to aspirate plasma cell aggregates retained in the marrow space by local reticulin fibrosis. The true percentage of plasma cells probably lies somewhere in between.

There remains a need to develop more objective and accurate methods of quantifying plasma cells to improve the diagnostic accuracy and, hence, management of patients with plasma cell dyscrasias. In the interim, we suggest the use of

MUM1 immunostain for quantification of plasma cells in myeloma patients as it mitigates the degree of over-estimation.

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1. Gabriel J, McGovern A, Robinson S, *et al*. A systematic study comparing aspirate versus trephine for quantifying plasma cell infiltration in newly-diagnosed myeloma. *Br J Haematol* 2016; 174: 818−20.
2. Lee N, Moon SY, Lee J-H, *et al*. Discrepancies between the percentage of plasma cells in bone marrow aspiration and BM biopsy: impact on the revised IMWG diagnostic criteria of multiple myeloma. *Blood* 2017; 7: e530.
3. Matsue K, Matsue Y, Kumata K, *et al*. Quantification of bone marrow plasma cell infiltration in multiple myeloma: usefulness of bone marrow aspirate clot with CD138 immunohistochemistry. *Hematol Oncol* 2017; 35: 323−8.
4. Stifter S, Babarović E, Valković T, *et al*. Combined evaluation of bone marrow aspirate and biopsy is superior in the prognosis of multiple myeloma. *Diagn Pathol* 2010; 5: 30.
5. Al-Quran SZ, Yang L, Magill JM, *et al*. Assessment of bone marrow plasma cell infiltrates in multiple myeloma: the added value of CD138 immunohistochemistry. *Hum Pathol* 2007; 38: 1779−87.
6. Julie D, Roland DW, Marie-Françoise D, *et al*. Comparison of bone marrow aspirate and bone marrow biopsy in the workup of patients with multiple myeloma. *Clin Lymphoma Myeloma Leuk* 2015; 15: e117.
7. Joshi R, Horncastle D, Elderfield K, *et al*. Bone marrow trephine combined with immunohistochemistry is superior to bone marrow aspirate in follow-up of myeloma patients. *J Clin Pathol* 2007; 61: 213−6.
8. Rajkumar SV, Dimopoulos MA, Palumbo A, *et al*. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol* 2014; 15: e538−48.
9. Ginsburg N. Numerosity estimation as a function of stimulus organization. *Perception* 1991; 20: 681−6.
10. Cousins JB, Ginsburg N. Subject correlation and the regular-random numerosity illusion. *J Gen Psychol* 1983; 108: 3−10.

EBV positive mucocutaneous ulcer with plasmacytic/plasmablastic differentiation and *MYC* rearrangement: a diagnostic challenge and a mimic of plasmablastic lymphoma

Sir,
Epstein−Barr virus positive mucocutaneous ulcer (EBVMCU) is a provisional entity in the 2017 World Health Organization (WHO) classification of lymphoid neoplasms. It is defined as a localised, indolent lesion occurring in cutaneous or mucosal sites in patients with immunosuppression including age-related, human immunodeficiency virus (HIV)-related, or iatrogenic such as those receiving immunosuppressants for transplantation and autoimmune diseases, or cancer patients receiving chemotherapy.[1−3] Histologically, there is a dense polymorphic infiltrate comprising a substantial number of large transformed B-cells, often with Hodgkin/Reed−Sternberg-like features that are EBV positive and mostly CD20 positive, usually accompanied by a band-like infiltrate of reactive T-cells. Plasmablastic lymphoma (PBL) is an aggressive lymphoma comprising a diffuse proliferation of large cells resembling B immuno-blasts or plasmablasts that are negative for CD20/PAX5 and positive for CD138/MUM-1 with a plasmacytic immuno-phenotype. PBL occurs most commonly in the oral cavity of HIV patients or in those with other causes of immunodeficiency. EBV infection is positive in about 60−75% of PBL cases. Furthermore, *MYC* rearrangement occurs in around half of the cases with PBL, and this genetic alteration is more common in EBV positive than EBV negative cases.[4] EBV is rarely positive in plasmacytoma, either in immunocompromised or immunocompetent patients. Herein we present a case with rheumatoid arthritis and long-term use of methotrexate and prednisolone who developed a EBVMCU in the oral cavity. Interestingly, the EBV positive large cells were CD20 negative, *MYC* rearranged with monoclonal plasmacytic/plasmablastic cells. Although our case resembled PBL or plasmacytoma, the lesion was localised and completely regressed after reduction of the doses of immunosuppressants without chemotherapy or radiation therapy, supporting the diagnosis of EBVMCU. This case illustrated that EBVMCU may be of plasmablastic morphology/immunophenotype and there is a grey-zone area between EBVMCU and PBL. Nevertheless, clinical and pathological correlation is essential as highlighted in the current case report.

A 72-year-old woman had a history of rheumatoid arthritis for at least 8 years and was under treatment with methotrexate (10 mg per week), prednisolone (10 mg per day) and hydroxychloroquine since January 2010. She developed a 2×1 cm, well-demarcated ulcer with peripheral elevation in her right lower lingual gingiva near the mouth floor (Fig. 1A). The incisional biopsy in May 2018 showed mucosal ulceration and a dense polymorphic infiltrate composed of medium-sized lymphoid cells admixed with many plasma cells (ranging from mature, atypical plasma cells to occasional plasmablasts), some neutrophils, and histiocytes (Fig. 1B). Some large atypical cells with vesicular nuclei and irregular nuclear membrane were noted (Fig. 1C,D). Mitotic figures were occasionally seen. Immunohistochemically, the large lymphoid cells expressed CD19, with a partial expression of CD30 and bcl-2, but not CD10, CD20, CD56, bcl-6, cyclin D1, or HHV-8 (Fig. 2). The proliferation index by Ki-67 was 80% (Fig. 2F). Around 30% of cells were marked by MYC (clone EP121, also known as Y69; Epitomics, USA) and this result was considered negative using the current cut-off criterion of 40% as used in diffuse large B-cell lymphoma. There was a moderate amount of CD3 positive reactive T-cells in the background and many plasmacytic cells highlighted by CD138 and IRF4/MUM-1 (Fig. 2D,E). These plasmacytic cells were monotypic for lambda light chain expression (Fig. 2G). *In situ* hybridisation for EBV-encoded small RNA was positive, mainly in the larger cells (Fig. 2H). Polymerase chain reaction-based clonality study showed a clonal *IGH* rearrangement. Fluorescence *in situ* hybridisation using paraffin section with Vysis break-apart probes revealed *MYC* rearrangement which was positive in 19% of cells (Fig. 2I),