A systematic study comparing aspirate *versus* trephine for quantifying plasma cell infiltration in newly-diagnosed myeloma

Bone marrow plasma cell infiltration (PCI), as measured by aspirate morphology and/or trephine immunohistochemistry, has long been an integral element in the diagnosis of plasma cell myeloma (PCM). According to the updated criteria of the International Myeloma Working Group (IMWG), demonstration of clonal bone marrow plasma cells ≥10% by either aspirate or trephine biopsy is required in addition to a 'myeloma defining event' for the diagnosis of PCM (Rajkumar *et al*, 2014). However, bone marrow sampling by aspirate or trephine frequently produces significantly different estimations of the level of PCI, with the trephine usually yielding an appreciably higher percentage (Joshi *et al*, 2008). There are a number of possible reasons for this, particularly the tendency for myeloma to exhibit a patchy distribution of plasma cell deposits in the bone marrow, in addition to aspiration failure of plasma cells due to niche adherence or fibrosis (Terpstra *et al*, 1992). In such cases, it is recommended that the higher infiltration value be used diagnostically (Rajkumar *et al*, 2001). However, failure to obtain either an adequate aspirate or trephine sample is not uncommon in the work-up of new patients, and therefore to rely one result in the absence of the other may lead to spurious estimations of the PCI.

Given the frequent disparity between aspirate and trephine PCI values, we sought to investigate whether mathematical modelling might allow reasonable prediction of one value based on the other. In a single-institution retrospective study, we reviewed 159 patients of whom only 87 (54·7%) had both aspirate and trephine results available. These results were used to model and quantify the relationship between aspirate and trephine estimations in order to determine if the two methods produce concordant data. To our knowledge, this is the first time that such a modelling approach has been used to study this neglected but important issue in PCM.

A 6-year retrospective analysis of 210 PCM patients presenting to the Royal Sussex County Hospital in Brighton, UK between January 2008 and December 2013 was carried out. Data from the diagnostic bone marrow biopsy was available for 159 patients, of whom 87 (54·7%) had both aspirate and trephine samples analysed and PCI levels recorded. These

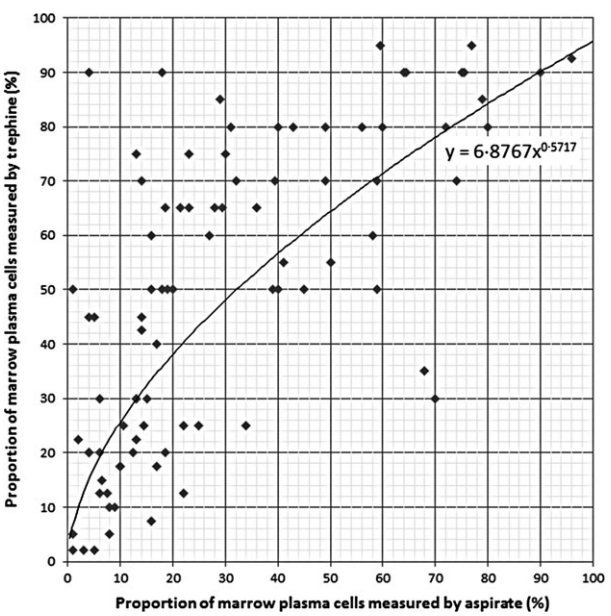

Fig 1. Scatter graph plot showing plasma cell infiltration results for paired aspirate and trephine samples (expressed relative to total nucleated cells). A best-fit curve is shown with equation $y = 6{\cdot}88x^{0{\cdot}57}$ (R^2 value of 0·461).

Table I. Stratification of plasma cell infiltration results for paired aspirate and trephine samples according to discrete per cent ranges (expressed relative to total nucleated cells).

	Stratification of results into discrete ranges based on the aspirate sample only				
	Range (%)	≤10	11–30	31–60	>60
Stratification of results into discrete ranges based on the trephine biopsy and expressed relative to the aspirate sample (%)	≤10	33	3	0	0
	11–30	48	36	5	8
	31–60	14	27	35	8
	>60	5	33	60	85
	Total	100%	100%	100%	100%

were determined by standard morphology of May-Grünwald-Giemsa stained aspirate samples or immunohistochemical staining for CD138-positive cells in trephine core biopsies. In both cases, this was expressed as a percentage of plasma cells relative to total nucleated cells.

Bone marrow aspirate *versus* trephine PCI data were plotted on a scatter-graph for all 87 patients (Fig 1) and analysed using SPSS (IBM Corp., Armonk, NY). This reveals that the aspirate and trephine consistently generate discordant results, with the aspirate samples generally yielding a significant underestimate compared with the trephine. This is particularly prominent at the lower levels of PCI: in patients with aspirate levels of 10% or less, usually indicative of monoclonal gammopathy of undetermined significance (MGUS), 67% had trephine levels of greater than 10% infiltration, with 14% having 30–60% infiltration and 5% having over 60% infiltration. By contrast, at higher levels of PCI there is greater concordance between the aspirate and trephine levels, with 85% of aspirate samples over 60% infiltration in agreement with the trephine samples.

Using best-fit curve analysis of the data ($R^2 = 0{\cdot}461$), we derived two equations that mathematically relate the datasets:

$$\text{trephine} = 6{\cdot}88 \times (\text{aspirate})^{0{\cdot}57}$$

$$\text{aspirate} = (\text{trephine}/6{\cdot}88)^{1{\cdot}75}$$

These equations permit a theoretical derivation of an unknown aspirate sample based on a known trephine result, or vice versa. Table I shows the results stratified for paired aspirate/trephine samples according to PCI values within discrete and clinically significant ranges of below 10%, 10–30%, 30–60% and >60%.

Our model comparing PCI levels between paired aspirate and trephine samples in newly diagnosed PCM patients clearly yields a non-linear relationship between the two. The best-fit curve demonstrates a strong tendency for the aspirate to significantly underestimate the PCI percentage of the trephine at the lower range (with only 33% of samples identified on both aspirate and trephine as having an infiltration of ≤10%) while becoming progressively similar at the higher ranges (85% of samples identified on both aspirate and trephine as having infiltration >60%). This finding highlights the importance of obtaining both aspirate and trephine results for the purpose of diagnostic myeloma work-up. However, in the absence of one or other result, the conversion equations presented here may allow a reasonable estimate of the missing measurement. This may be useful for monitoring a patient's response to therapy, for instance when comparing a trephine sample at diagnosis with an aspirate sample post-treatment, or vice versa, or for comparing trephine data for one patient group with aspirate data for another patient group.

The conversion equations also highlight the fact that arbitrary thresholds of PCI considered important for diagnosis of PCM are unsuitable for both aspirate and trephine samples. For example, PCI of 60% by trephine (a myeloma treatment defining event according to the IMWG; Rajkumar *et al*, 2014) would equate to a level of 44% by aspirate. Similarly, PCI of 30% by trephine (myeloma major criteria according to previous World Health Organization criteria; Jaffe *et al*, 2001) would equate to a level of only 13% by aspirate. Strikingly, a PCI of 10% by trephine (required for any diagnosis of PCM) would equate to a level of only 2% by aspirate, in-keeping with the observation that many cases of PCM have apparently 'normal' bone marrow aspirate results.

Our data and modelling reveal the relationship between aspirate and trephine PCI levels and highlight the fact that both results are required for a proper work-up of a newly presenting PCM patient. The consistent underestimation of PCI by aspirate compared with trephine suggests that this might have significant impact on a patient's diagnosis, risk stratification, treatment options and ultimate outcome. In particular, for patients having only an aspirate and no trephine sample, we believe that higher risk patients may be inadvertently undertreated, and that some cases of PCM may be completely misdiagnosed as MGUS. We propose that diagnostic criteria and treatment algorithms in PCM that depend upon certain arbitrary PCI thresholds should stipulate the mode by which the assessment is performed.

Author contributions

TC and AM designed the research study; JG and SR collected the data; AM analysed the data; JG, AM and TC wrote the paper; DW performed a critical review of the manuscript; all authors approved the final version of the paper.

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Long-term survival of acute promyelocytic leukaemia patients treated with arsenic and retinoic acid

Recently, treatment with arsenic and all-trans retinoic acid (ATRA) combined with or without chemotherapy has been shown to result in excellent outcomes for patients with newly diagnosed acute promyelocytic leukaemia (APL) (Estey *et al*, 2006; Hu *et al*, 2009; Iland *et al*, 2012; Lo-Coco *et al*, 2013; Zhu *et al*, 2013; Zhu & Huang, 2014). The National Comprehensive Cancer Network has adopted ATRA and arsenic trioxide (ATO) as first-line treatments for APL in its 2014 guidelines (O'Donnell *et al*, 2013). For non-high-risk APL patients [i.e., those with a white blood cell (WBC) $\leq 10 \times 10^9$ count/l], treatment with arsenic and ATRA without chemotherapy resulted in 2-year overall survival (OS) rates of 99% (Lo-Coco *et al*, 2013). Our group previously reported the results of a randomized trial (APL07 trial) comparing the oral administration of arsenic (Realgar-*Indigo naturalis* formula, RIF) and ATRA with i.v. administration of

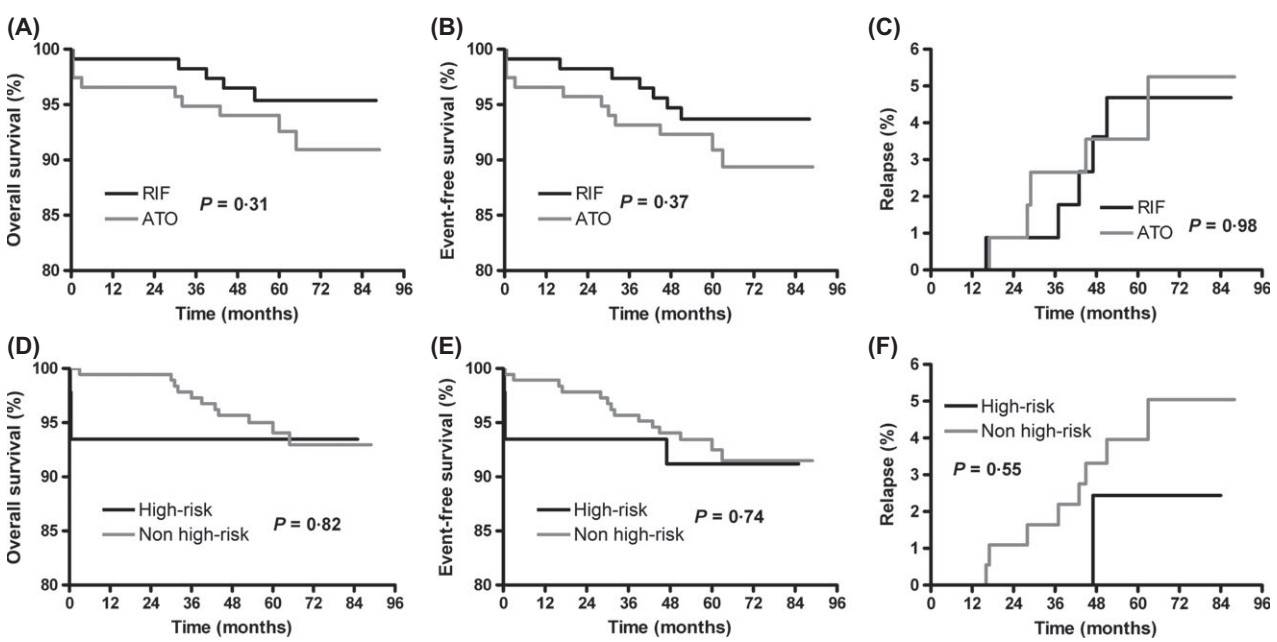

Fig 1. Survival and relapse in patients with acute promyelocytic leukaemia according to the APL07 treatment protocol. High-risk was defined as a white blood cell count $>10 \times 10^9$/l and non-high-risk is defined as a white blood cell count $\leq 10 \times 10^9$/l. RIF, Realgar-*Indigo naturalis* formula; ATO, arsenic trioxide.