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Letter to the Editor

Impact of the plasma cell quantification method on the International Myeloma Working Group diagnostic criteria for MGUS/SMM

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Running title: Plasma cell quantification methods for SMM/MGUS

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AU and KM initiated, designed, and performed the study, and wrote the manuscript. AU performed data collection, PC quantification, and statistical analyses. RM and DI prepared the pathology specimens and performed the digital analysis. The other authors managed patient care and collected the samples. All the authors critically reviewed and approved the final version of the manuscript.

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To the Editor,

Accurate differentiation between monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM) is crucial to stratify patient risk of progression and appropriate follow-up. The International Myeloma Working Group (IMWG) recommends making this distinction primarily based on the percentage of plasma cells (PCs) in the bone marrow (BM), using the highest value when both aspirate and core biopsy specimens are available[1]. However, multiple studies have demonstrated that PC percentages are consistently higher in biopsy samples compared with aspirate samples.[2-5]

These findings suggest that the MGUS and SMM diagnosis can be substantially affected by BM sample types. However, prior studies are limited by assessment of small area of BM smear or biopsy specimens, and were potentially influenced by observer bias. To address this, we compared PC percentages across different BM sample types using whole-slide imaging (WSI), enabling the unbiased quantification of a large number of cells populations.

We retrospectively identified patients with monoclonal gammopathy diagnosed at Kameda Medical Center or Kanazawa University Hospital between August 1, 2006, and February 28, 2025 who did not meet the IMWG diagnostic criteria for multiple myeloma (MM). Exclusion criteria included concurrent lymphoma and unavailability of BM samples. High-risk SMM was defined as ≥2 risk factors according to the IMWG 2/20/20 model,[6] using the percentage from BM aspirate specimens. Written informed consent was obtained from all patients at Kameda Medical Center; Kanazawa University Hospital followed an opt-out procedure per institutional guidelines. This study was approved by both institution's ethics boards, and was conducted in accordance with the Declaration of Helsinki.

BM smears were assessed by manually on May-Giemsa-stained BM slides. Whereas, CD138-immunostained clots and biopsies were digitized via a high-resolution scanner (Philips, Best, Netherlands) and analyzed with QuPath software (version 0.5.1; University of Edinburgh, Edinburgh, Scotland). The parameters for analysis and CD138-positive cell quantification methods are shown in **Supplemental Table 1** and **Supplemental Figure 1,** respectively. Samples were excluded from the analysis if the total number of detected cells was < 500 in clot specimens or < 3,000 in biopsy specimens.

In the prognostic analysis, patients with high-risk SMM or systemic AL amyloidosis were excluded. The progression-free rate (PFR) was defined as the proportion of patients who

did not progress at a pre-specified time point, and the progression-free survival (PFS) was defined as the time from diagnosis to progression or death from any cause. Cumulative incidence curves were compared using Gray test. Continuous and categorical variables were analyzed with paired t-tests and McNemar's test. The areas under the time-dependent ROC curves were compared using iid-based Wald test. Categorical net reclassification improvement (NRI) was used to compare the risk stratification models, and 95% confidence intervals and P-values were obtained using 2,000 bootstrap replications. All calculations were performed using R version 4.4.1 (R Foundation, Vienna, Austria). Statistical significance was set at $P<0.05$.

Overall, 405 patients were identified retrospectively. Of these, 25 met the exclusion criteria and 380 were therefore included in the final analysis (**Supplemental Figure 2**). Two or more sample types (smear, biopsy, and clot) were available for 363 patients (95.5 %), whereas all three were available for 197 patients (51.8%). At baseline, 8 and 55 patients had high-risk SMM and systemic AL amyloidosis, respectively. In 82 patients, biopsy specimens were unavailable because of inadequate sampling, insufficient cellularity, or the physician's decision to omit biopsy. Baseline characteristics did not differ significantly between patients with and without biopsy specimens (**Table 1**).

In cases with all three sample types available, the percentage of PCs differed significantly across smear (median 2.4%, IQR: 1.2–4.6), biopsy (median 12.8%, IQR: 8.7–20.9), and clot (median 14.8%, IQR: 9.2–23.3) specimens (**Figure 1, A–D**)**.** When patients were classified as having MGUS or SMM using a uniform PC percentage cutoff of 10% for each sample type, the proportion classified as MGUS was 90.4% by smear, 35.0% by biopsy, and 27.4% by clot, with significant differences across specimen types (smear vs. biopsy, $P < 0.001$; biopsy vs. clot, $P = 0.041$; **Figure 1, E**). Furthermore, two patients classified as MGUS or SMM by smear were reclassified as MM based on having a PC percentage ≧60% in biopsy or clot specimens. A weak negative correlation was observed between the absolute difference in PC percentage (biopsy–smear) and the absolute nucleated cell count (ANC) of BM aspirate ($R = -0.278$, $P < 0.001$; **Figure 1, F**). The difference was significantly greater in patients with a low ANC (<100×10^3 /μL; median 10.5%) than in those with a high ANC (≧100×10^3 /μL; median 6.1%; $P < 0.001$; **Figure 1, G**). The PC percentage in the biopsy and clot specimens further showed a strong correlation ($R = 0.682$, $P < 0.001$) and the median clot-biopsy difference in PC percentage was 1.59% (IQR: -3.32 to 5.9).

In the ROC curve analysis, no significant differences in the areas under the curve (AUC) were found across the three specimen types for PC percentage when the 3-year PFR and the 3-year PFS rate were used as outcomes (PFR: smear, 0.78; biopsy, 0.70; clot, 0.78; PFS rate: smear, 0.69; biopsy, 0.68; clot, 0.59; **Figure 1, H–I**). The optimal PC percentage cutoff values for stratifying 3-year PFR were 3.0% (sensitivity, 0.786; specificity, 0.664), 14.9% (sensitivity, 0.811; specificity, 0.683), and 23.9% (sensitivity, 0.605; specificity, 0.849) in smear, biopsy, and clot specimens, respectively.

To evaluate the prognostic performance of these sample type-specific cutoff values, we compared the cumulative incidence of disease progression between patients with high and low tumor burden. All three cutoff values significantly stratified prognosis, with a 3-year cumulative incidence of 12.7% vs. 2.3% ($P < 0.001$) in smears, 9.2% vs. 1.4% ($P = 0.023$) in biopsy specimens, and 19.4% vs. 2.9% ($P < 0.001$) in clot specimens (**Figure 2, A–C**). In contrast, applying a uniform 10% cut-off value across specimen types achieved relatively weaker stratification, particularly for biopsy specimens (3-year cumulative incidence of disease progression: smears, 26.2% vs. 5.1% [$P < 0.001$]; biopsy specimens, 5.2% vs. 2.3% [$P = 0.22$]; and clot specimens, 7.7% vs. 1.9% [$P = 0.028$]; **Figure 2, D–F**). An NRI-based comparison of discriminative performance between each model for the 3-year PFR showed significantly better stratification by our sample type–specific cutoff model in biopsy specimens (NRI = 0.286; 95% CI, 0.206–0.359; $P < 0.001$). A similar trend was observed in smear and clot specimens, although not reaching statistically significance (smear: NRI = 0.231; 95% CI, −0.038 to 0.510; $P = 0.099$; clot: NRI = 0.225; 95% CI, −0.125 to 0.522; $P = 0.17$).

This study demonstrated that the PC percentage differed markedly across BM smear, biopsy, and clot specimens, and could substantially affect the diagnosis of MGUS and SMM. Moreover, our findings suggest that optimal cutoff values for PC percentage differ by sample type for prognostic stratification, and that sample type-specific cutoff values may enable more accurate risk stratification of patients with MGUS/SMM than a uniform 10% cutoff value.

Our finding that the PC percentage is lower in smears is consistent with previous reports[2-5]. This discrepancy can be explained by hemodilution of BM aspirates[7], non-uniform distribution of myeloma cells[8], BM fibrosis[9], and a lack of objectivity in the area selected. A strength of our study is addressing the limitations of previous reports, such as the small number of cells evaluated and field selection bias, by using WSI-based quantification. Although

there was no significant difference in the AUC of the ROC curves, WSI-based quantification is expected to provide a more accurate assessment that overcomes the aforementioned challenges of smear-based evaluation. Furthermore, the significant diagnostic disparities between sample types underscore the need for a clear description of the BM sample type used to diagnose MGUS/SMM in clinical studies. The strong correlation between clots and biopsies suggests that in certain settings, clot analysis could serve as a practical alternative to biopsy, particularly for monitoring disease progression or response.

This study has several limitations. First, the modest sample size, short follow-up, and lack of an independent validation cohort limit the strength of the conclusions. Second, selection bias cannot be excluded because patients with higher M-protein levels may have been more likely to undergo BM examination. Third, although digital analysis improves objectivity, its accuracy can be affected by staining quality, distribution of the PCs and the total number of cells analyzed. Nevertheless, prior studies have demonstrated accuracy in comparison with manual counting.[10,11] The lack of consensus regarding digital image analysis methods, including the software used and parameters that require partial manual adjustment, is also a limitation, as we cannot exclude the possibility of measurement variability due to technical factors. Standardization of analytical protocols is required. Despite these limitations, our findings highlight the importance of specimen type in the diagnosis of MGUS/SMM and suggest adopting sample type-specific cut off values in future revisions of the IMWG diagnostic criteria.

In conclusion, our results show that BM PC percentage assessment in monoclonal gammopathy varies significantly according to the BM specimen type, while WSI-based clot analysis yields results comparable to those of biopsies and may serve as a less invasive and repeatable alternative. Specimen type-specific thresholds improve MGUS/SMM classification and prognostication over the conventional 10% cutoff. Prospective multicenter studies with longer follow-up periods are needed to validate these recommendations and assess their impact on patient management.

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Table 1. Clinical characteristics of the enrolled patients.

Variables	Overall n = 380	Biopsy available n = 298	Biopsy unavailable n = 82	P value
Median age, years (IQR)	71 (63-78)	71 (63-78)	72 (64-78)	0.39
Female sex, n (%)	161 (42.4)	133 (44.6)	29 (35.4)	0.17
Heavy chain, n (%)				0.73
IgG	244 (64.2)	193 (64.8)	51 (62.2)	
IgA	71 (18.7)	53 (17.8)	18 (22.0)	
IgM	19 (5.0)	13 (4.4)	6 (7.3)	
Light chain only	37 (9.7)	31 (10.4)	6 (7.3)	
Others	9 (2.4)	8 (2.7)	1 (1.2)	
Light chain, n (%)				0.77
Kappa	202 (53.2)	156 (52.3)	46 (56.1)	
Lambda	176 (46.3)	140 (47.0)	36 (43.9)	
Others	2 (0.53)	2 (0.67)	0	
Laboratory parameters, median (IQR)				
TP (g/dL)	7.2 (6.5-7.8)	7.2 (6.5-7.8)	7.2 (6.5-7.9)	0.93
Alb (g/dL)	3.9 (3.3-4.3)	3.9 (3.3-4.3)	4.0 (3.4-4.2)	0.62
LDH (U/L)	193 (166-230)	194 (167-229)	189 (163-233)	0.89
Involved Ig (mg/dL)	1,518 (968-2,235)	1,556 (1,021-2,238)	1,381 (781-2,014)	0.24
Involved FLC (mg/L)	55.9 (26.5-155.8)	57.0 (26.5-166.6)	47.7 (27.4-107.5)	0.18
Serum β2MG (mg/dL)	2.6 (1.8-4.2)	2.6 (1.8-4.1)	2.6 (1.79-4.2)	0.84
Total cells analyzed, median (IQR)				
Biopsy	23,647 (12,119-36,740)	-	-	

Clot	12,578 (3,800-35,613)	8,467 (2,481-29,785)	18,503 (5,479-38,732)	0.28

Abbreviations: TP; total protein, Alb; albumin, LDH; lactate dehydrogenase, Ig; immunoglobulin, FLC; free light chain, β2MG; beta−2 microglobulin.

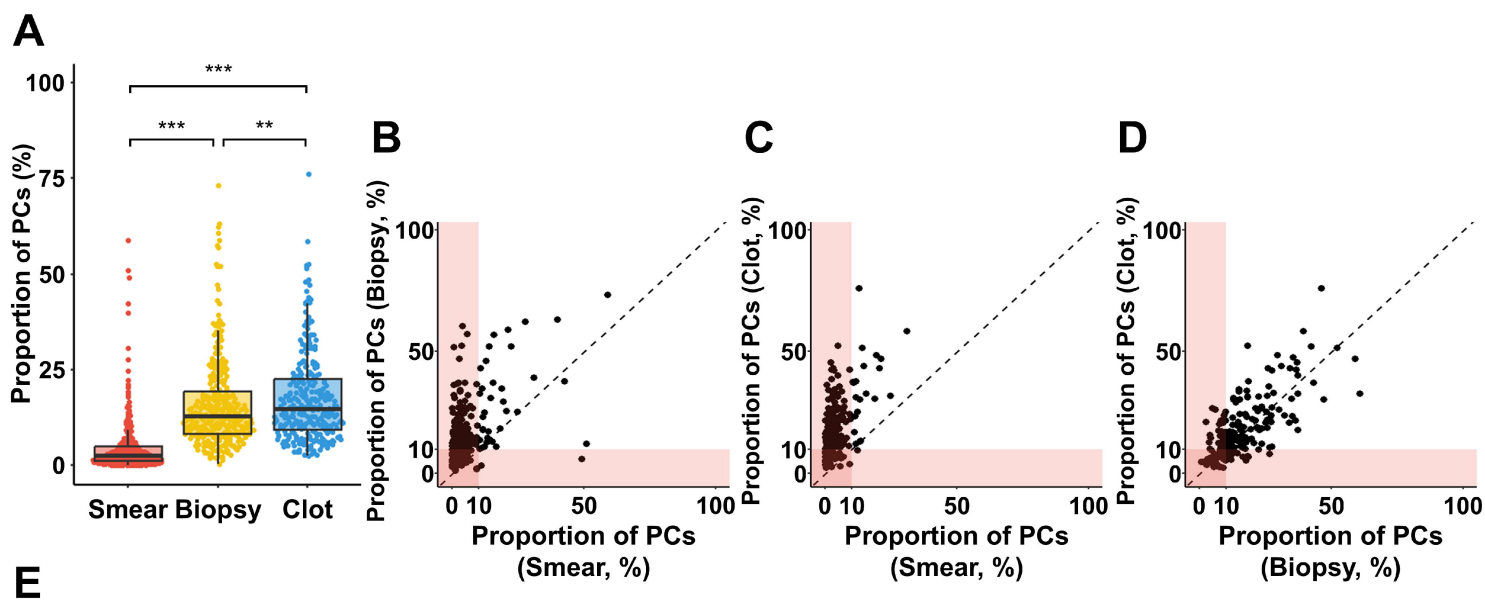
Figure Legends

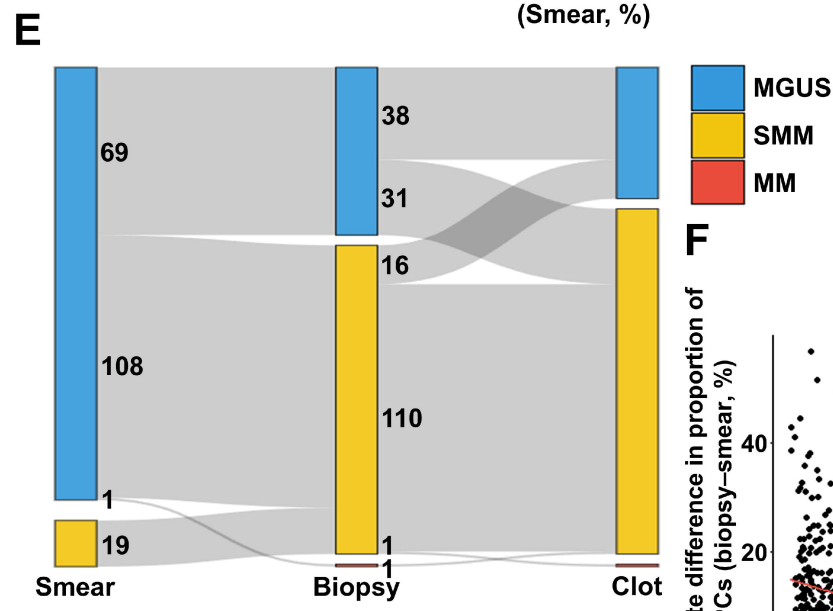
Figure 1. Specimen-dependent differences in bone marrow plasma cell percentage (PC%). (A) Distribution of PC% across the three specimen types. (B–D) Pairwise comparisons of PC% between specimen types shown as scatter plots, where dashed lines indicate the line of identity (y = x). (E) Sankey diagram with table summarizing the case classification in each specimen using the uniform 10% IMWG cut-off and how classifications shift across specimen types. (F) Scatter plot of the absolute difference in PC% between the biopsy and smear versus the absolute nucleated cell count (ANC) of the smear. (G) Comparison of the absolute difference in PC% between the biopsy and smear, stratified by high- and low-ANC smear groups. (H) Time-dependent ROC curves for the 3-year progression-free rate using PC% for each specimen type. (I) Time-dependent ROC curves for the 3-year progression-free survival using PC% for each specimen type.

Abbreviations: PC; plasma cell, MGUS; monoclonal gammopathy of undetermined significance, SMM; smoldering multiple myeloma, MM; multiple myeloma, ANC; absolute nucleated cell count, PFR; progression-free rate, PFS; progression-free survival. BM; bone marrow

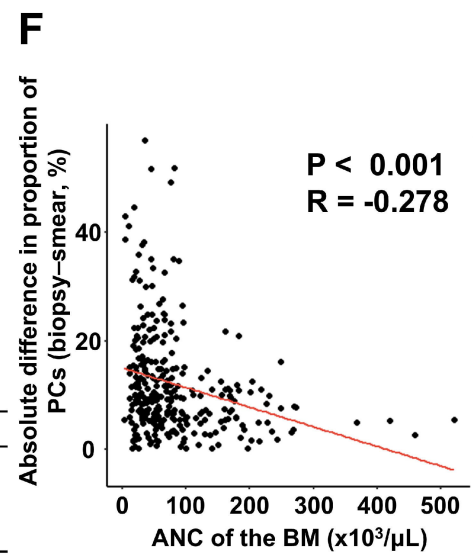
Figure 2. Cumulative incidence of disease progression based on sample specific cut-off (Smear 3.0%, Biopsy 14.9%, Clot 23.9%) and the conventional 10% cut-off.

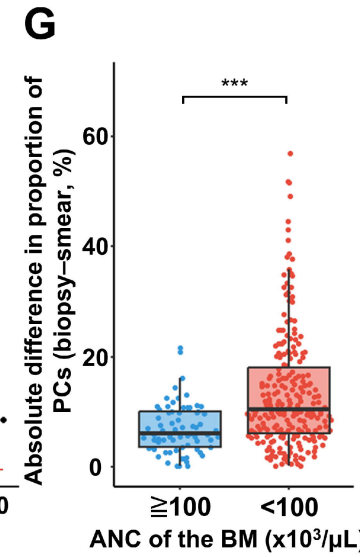
Abbreviations: TB; tumor burden.

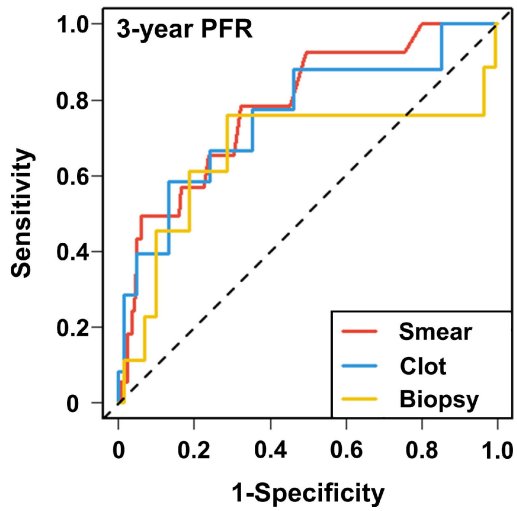


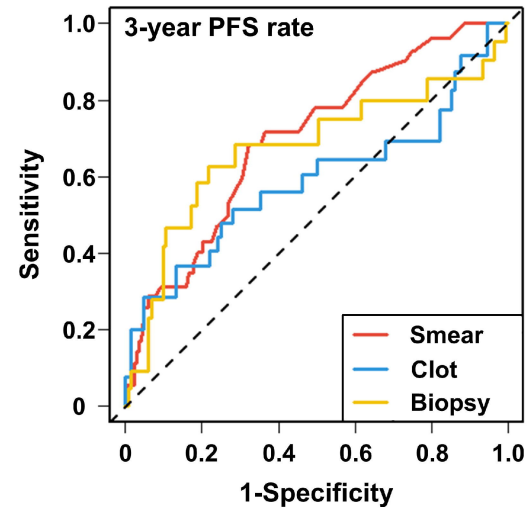


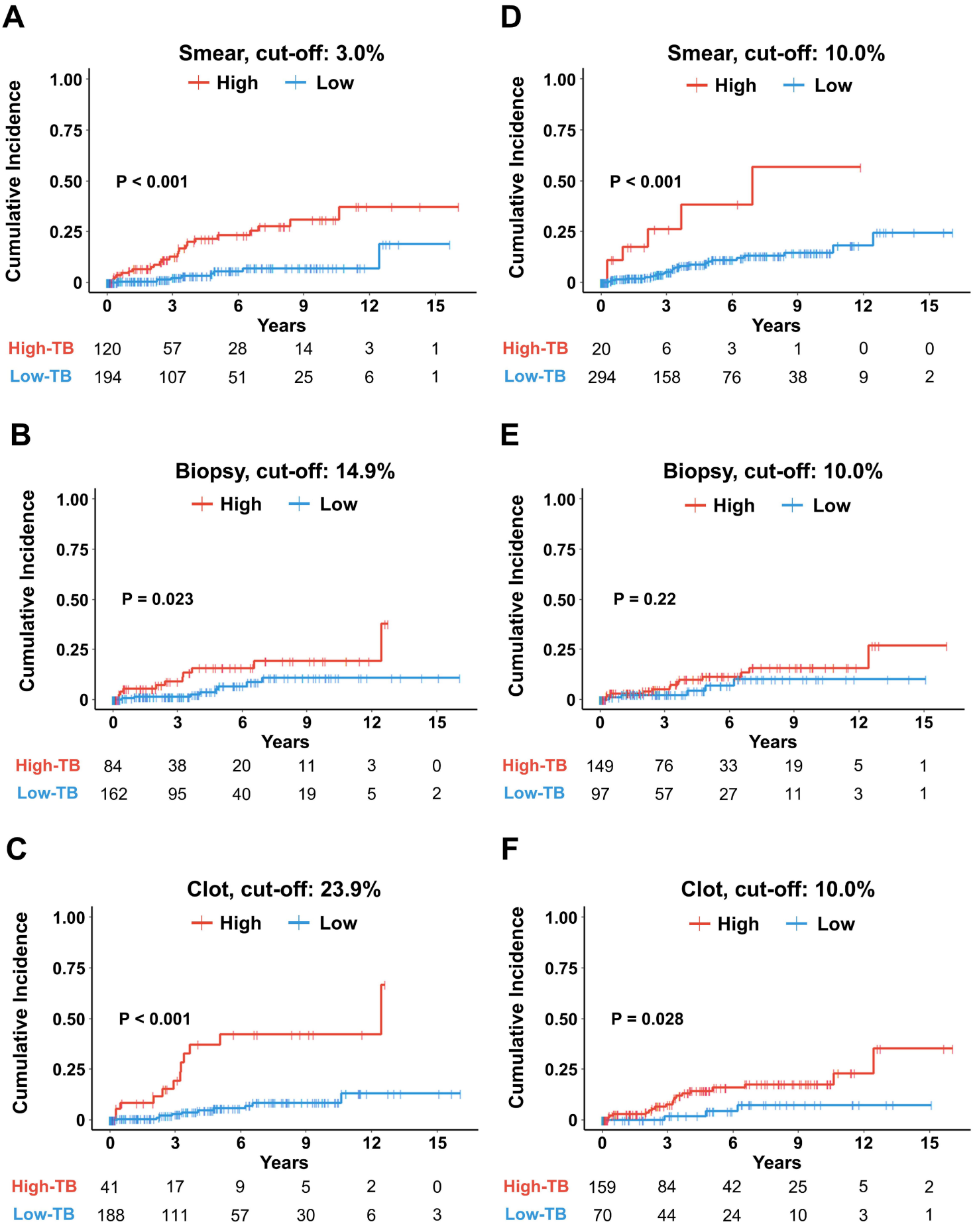
	MGUS	SMM	MM
Smear	178 (90.4%)	19 (9.6%)	0
Biopsy	69 (35.0%)	127 (64.5%)	1 (0.5%)
Clot	54 (27.4%)	142 (72.1%)	1 (0.5%)









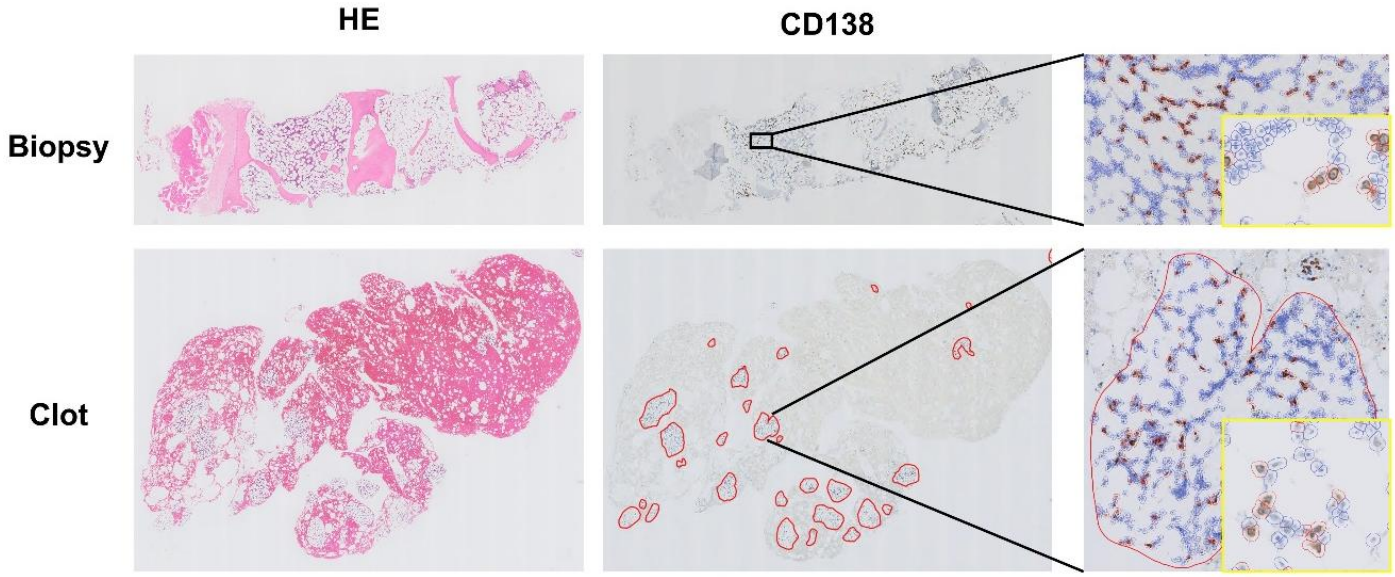

A
Smear, cut-off: 3.0%
High
Low
Cumulative Incidence
1.00
0.75
0.50
0.25
0
P < 0.001
0
3
6
9
12
15
Years
High-TB 120 57 28 14 3 1
Low-TB 194 107 51 25 6 1
D
Smear, cut-off: 10.0%
High
Low
Cumulative Incidence
1.00
0.75
0.50
0.25
0
P < 0.001
0
3
6
9
12
15
Years
High-TB 20 6 3 1 0 0
Low-TB 294 158 76 38 9 2
B
Biopsy, cut-off: 14.9%
High
Low
Cumulative Incidence
1.00
0.75
0.50
0.25
0
P = 0.023
0
3
6
9
12
15
Years
High-TB 84 38 20 11 3 0
Low-TB 162 95 40 19 5 2
E
Biopsy, cut-off: 10.0%
High
Low
Cumulative Incidence
1.00
0.75
0.50
0.25
0
P = 0.22
0
3
6
9
12
15
Years
High-TB 149 76 33 19 5 1
Low-TB 97 57 27 11 3 1
C
Clot, cut-off: 23.9%
High
Low
Cumulative Incidence
1.00
0.75
0.50
0.25
0
P < 0.001
0
3
6
9
12
15
Years
High-TB 41 17 9 5 2 0
Low-TB 188 111 57 30 6 3
F
Clot, cut-off: 10.0%
High
Low
Cumulative Incidence
1.00
0.75
0.50
0.25
0
P = 0.028
0
3
6
9
12
15
Years
High-TB 159 84 42 25 5 2
Low-TB 70 44 24 10 3 1

Supplementary Data

Supplemental Table 1. Detailed parameter settings for digital analysis of whole-slide images in QuPath.

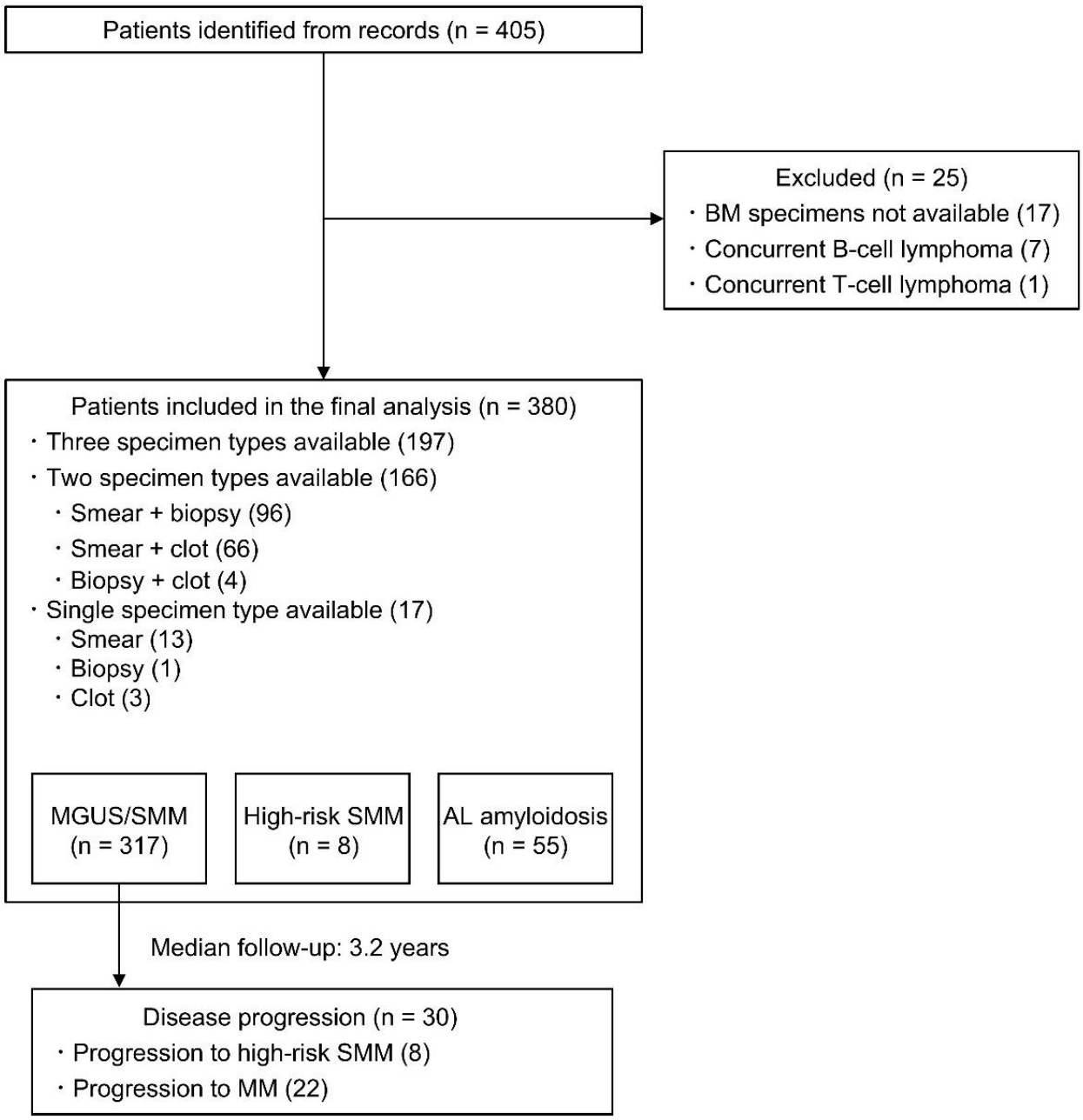
Setup parameters	
Detection image	Optical density sum
Requested pixel size	0.5μm
Nucleus Parameters	
Background radius	8μm
Use opening by reconstruction	Enabled
Median filter radius	0μm
Sigma	1.5μm
Minimum area	5μm^2
Maximum area	400μm^2
Intensity parameters	
Threshold	0.03-0.20 (manually adjusted for each sample)
Max background intensity	2
Split by shape	Enabled
Exclude DAB (membrane staining)	Disabled
Cell parameters	
Cell expansion	5μm
Include cell nucleus	Enabled
General parameters	
Smooth boundaries	Disabled
Make measurements	Enabled
Intensity threshold parameters	
Score compartment	Cell: DAB OD max
Threshold 1+	0.10-0.50 (manually adjusted for each sample)
Single threshold	Enabled

Supplemental Figure 1. Digital cell detection in QuPath.



Abbreviations: HE; hematoxylin-eosin staining.

Supplemental Figure 2. Flow-chart of patient selection.



Abbreviations: BM; bone marrow, MGUS; monoclonal gammopathy of undetermined significance, SMM; smoldering multiple myeloma.