Comparative Morphology of Myeloproliferative Neoplasms in Bone Marrow Trephine Specimens.

Dr Bridget S Wilkins
Consultant Haematopathologist
St Thomas’ Hospital, London
Myeloproliferative neoplasms in the 2008 WHO Classification

- Chronic myelogenous leukaemia, BCR-ABL1+
- Chronic neutrophilic leukaemia (CNL)
- Chronic eosinophilic leukaemia (CEL), n.o.s.
- CEL with PDGFRα, PDGFRβ or FGFR1 rearrangement
- Polycythaemia vera (almost all with JAK2 mutations)
- Essential thrombocythaemia (+/-JAK2, MPL mutations)
- Primary myelofibrosis (+/-JAK2, MPL mutations)
- Myeloproliferative neoplasm, unclassifiable
  - Systemic mastocytosis +/- associated clonal haematological non-mast cell lineage disease (KIT mutations etc.)
In the ‘genetic era’ is there a continuing role for histopathology?

• Yes - to establish MPN vs “other” in diagnosis, but:
  – Complex, maturing cell mix to evaluate rather than a fairly homogeneous clone in AML.
  – Prolonged clinical course, often with slow progression; varying proportions of haemopoietic cells are non-clonal.
  – Distinction of MPN from reactive conditions is a genuine and frequent difficulty.

• Is histopathology useful to subtype MPN?
CML, *BCR-ABL1*+ as a paradigm for molecular classification in MPN

- Little justification now for bone marrow examination in diagnosis
- Potential use as baseline for comparison with suspected accelerated and blastic phases subsequently
- Some patients present with disease mimicking essential thrombocythaemia – morphology helpful here to indicate need for *BCR-ABL1* testing
What CML looks like (chronic phase)
Is Polycythaemia Vera the next ‘molecular entity’ in MPN?

• More than 95% JAK2-V617F positive
• Additional examples positive for JAK2 exon 12 mutations
• Currently uncertain clinical relevance of JAK2-V617F allele burden but this may be becoming more clear with increasing quantitative measurement of the mutation
• (JAK2-V617F also found in cases of apparent true ET and PMF)
Polycythaemia Vera – is histology useful?

- Trilineage haemopoietic cell expansion, with prominent (not necessarily predominant) erythropoiesis.
- MKs show wide variation in size and morphology - rare “staghorn” nuclei but abundant “cloud-like” and small atypical MK variants. Clustering.
- Reticulin highly variable; if grade 4 (WHO score 3), consider post-PV myelofibrosis.
- PV with JAK2 exon 12 mutations may only have subtle abnormalities histologically.
PV - pan-myelosis, usually with a major erythroid component
Large megakaryocyte with ‘cloud-like’ nucleus, plus small atypical variants
PV with JAK2 exon 12 mutation rather than V617F (exon 14)

Erythroid expansion; little MK atypia
PV with JAK2 exon 12 mutation rather than V617F (exon 14)
Essential Thrombocythaemia and Primary Myelofibrosis – are we halfway to a molecular classification?

- **JAK2-V617F** in approximately 50%
- **MPL-W515K/L** and new variants in a further 3-5%
- **JAK2-V617F** homozygosity distinctly less frequent than in PV (exceptional in ET)
- No clear clinical correlations with molecular status
Essential Thrombocythaemia – is histology useful?

- Isolated increase in MK with abundant “staghorn” variants, absence of “cloud-like” and small atypical MK variants, plus reticulin no greater than grade 2 (WHO score = 0 or 1), all support a diagnosis of ET versus other Ph-negative MPN.

  (Be cautious - “staghorn” MK variants may be found in unrelated disorders.)

- Limited/loose clustering of MK.
Large megakaryocytes with ‘staghorn’ nuclei in ET
Primary Myelofibrosis –
Pre-fibrotic/early fibrotic subtype

- Trilineage haemopoietic cell expansion, typically (but not always) with granulocytes and megakaryocytes predominating.
- MK show wide variation in size and morphology - rare “staghorn” nuclei, abundant large and small atypical variants (including “cloud-like” nuclei).
- Prominent/tight clustering of MK.
- Reticulin up to grade 3 (WHO score = 0-2).
PMF is a granulo-megakaryocytic myelosis
Two men, mid-50s, similar haematology, no splenomegaly, plts 750-800
Early fibrotic stage PMF histology according to WHO criteria
Primary Myelofibrosis with established fibrosis

- Initially hypercellular; progression to reduced or absent haemopoietic activity.
- Marked stromal fibrosis (if BMT hypocellular, this may appear very pale; fat spaces may be retained).
- Neo-osteogenesis variable.
- Angular, hyperchromatic MK nuclei usually remain.
- Important to look for blast cells (& exclude alternative pathology such as metastasis).
Osteomyelofibrosis as end stage of PMF
ET versus pre-fibrotic PMF – histology is key…

- At most, minimal increase in age-adjusted cellularity in ET
- Predominantly megakaryocytic expansion
- Distinct minor/major population of large, hypermature MK with “staghorn” nuclei
- Other MK normal
- MK clustering only a minor feature
- At most, minimal increase in reticulin (grade 1-2; WHO score 0-1)
ET versus pre-fibrotic PMF – histology is key…

• But does it matter?
Practical considerations for assessing BM histology in MPN

- Major features that can be assessed with reasonable consistency:
  - Overall haemopoietic cellularity
  - Relative predominance of increased megakaryocyte numbers
  - Megakaryocyte distribution
  - (Megakaryocyte cytological details)
  - Reticulin pattern (and stromal inflammatory features)
Stromal reticulin patterns in trephine sections

Grade 1 = WHO 0
Grade 2 = WHO 0 or 1
Grade 3 = WHO 2
Grade 4 = WHO 3
PT-1 Trial: Subjective weights given to histology features in WHO Classification
Pre-fibrotic PMF? Is this really a different disease from ET?
Pre-fibrotic PMF? Is this really a different disease from ET?
Variation within a single BM core
(JAK2-V617F ?ET - F, 40 y.o., plts ~900)
PT-1: Overall survival by reticulin grade

Overall survival by reticulin grade over time from trial entry (years). The graph shows survival rates for different reticulin grade levels: Reticulin 0-1 (red), Reticulin 2 (black), Reticulin 3-4 (pink). The p-value for the multivariate analysis is 0.10.
PT-1: Myelofibrotic transformation by reticulin grade

Myelofibrosis-free survival vs. time from trial entry (years)

- Reticulin 0-1
- Reticulin 2
- Reticulin 3-4

p=0.0007 Multivariate
62-yr-old lady, persistent plts 600-800, other haematology and spleen normal, JAK2-V617F neg
42-yr-old man, incidental finding of plts 875, other haematology and spleen normal, JAK2-V617F neg.
50-yr-old lady, plts ~600 for 10 yrs, other haematology and spleen normal, JAK2-V617F equivocal.
80-yr-old lady. CML, *BCR-ABL1*+ve, diagnosed 18 months earlier. Slow response to imatinib. Platelets now 1100 (orig. 470)
What CML looks like (chronic phase)
10 years later… repeat bone marrow trephine in 2007
Bone marrow trephine 1997
(re-cut H&E in 2007)
So, does histopathology have a role in diagnosis of MPN?

- Yes, of course, but possibly a more selective one than advocated by WHO
- Under-appreciated that it can help to exclude alternative, reactive mimics
- Also has potential use in follow-up of these chronic neoplastic disorders over time
- The latter is likely to be important as new treatments are developed