The use of genetic tests to diagnose and manage patients with myeloproliferative and myeloproliferative/myelodysplastic neoplasms, and related disorders

A British Society for Haematology Good Practice Paper

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Methodology

This Good Practice Paper was compiled according to the BSH process at [https://b-s-h.org.uk/media/16732/bsh-guidance-development-process-dec-5-18.pdf]. The British Society for Haematology (BSH) produces Good Practice Papers to recommend good practice in areas where there is a limited evidence base but for which a degree of consensus or uniformity is likely to be beneficial to patient care. The Grading of Recommendations Assessment, Development and Evaluation (GRADE) nomenclature was used to evaluate levels of evidence and to assess the strength of recommendations. The GRADE criteria can be found at http://www.gradeworkinggroup.org.

Literature review details

Pubmed was searched from Jan 2018 – September 2020 using the terms (myeloproliferative OR polycythemia OR thrombocythemia OR myelofibrosis OR eosinophilia OR mastocytosis OR neutrophilia OR myelomonocytic OR eosinophilic CEL OR CNL or CMML or JMML) AND (mutation OR variant) AND (diagnosis OR prognosis). Summary information from the 1063 hits was manually reviewed to identify 135 relevant publications. Relevant studies prior to January 2018 were identified from reviews published during the literature search period.

Review of the manuscript

Review of the manuscript was performed by the BSH Guidelines Committee General Haematology Task Force, the BSH Guidelines Committee and the General Haematology sounding board of BSH. It was also on the members section of the BSH website for comment. It has also been reviewed by members of the National

51 Cancer Research Institute (NCRI) MPN subgroup, the Chair of the NCRI MDS
52 subgroup and lead scientists from the Genomics Laboratory Hubs in England and
53 representative genetic testing laboratories in Wales, Scotland and Northern Ireland;
54 these organisations do not necessarily approve or endorse the contents.
55
56 **Introduction**
57 Genetics and genomics are playing an increasingly important role in the diagnosis
58 and management of patients with haematological neoplasms. Next generation
59 sequencing (NGS) panels are widely available and initiatives such as the National
60 Genomic Test Directory (NGTD; www.england.nhs.uk/publication/national-genomic-
61 test-directories) in England along with parallel developments in the devolved nations
62 aim to facilitate a standardised approach to testing and provide equity of access. A
63 key component of this approach is the definition of eligibility criteria for specific tests
64 to ensure appropriate usage from both clinical and financial perspectives.
65
66 This good practice paper focuses on the use of genetic and genomic tests for adult
67 chronic myeloid neoplasms as defined by the World Health Organization (1),
68 including myeloproliferative neoplasms (MPN), myelodysplastic/myeloproliferative
69 neoplasms (MDS/MPN), myeloid/lymphoid neoplasms with eosinophilia and
70 rearrangement of *PDGFRA*, *PDGFRB*, or *FGFR1*, or with *PCM1-JAK2* (MLN-eo) and
71 mastocytosis. We have not included chronic myeloid leukaemia (CML) as this has
72 been covered recently elsewhere (2), as has the full spectrum of clinical and
73 laboratory investigations for patients with abnormal blood counts and/or suspected
74 myeloid neoplasia (1, 3-9).
75

Classical *BCR-ABL1*-negative myeloproliferative neoplasms

Screening investigations for erythrocytosis, thrombocytosis, suspected myelofibrosis and atypical thrombosis: Molecular screening investigations for the common MPN phenotype driver mutations (*JAK2*, *CALR*, *MPL*), usually performed on peripheral blood DNA, are shown in Table 1. These assays will identify a mutation in almost all patients with polycythaemia vera (PV) and 85–90% with essential thrombocythaemia (ET) and primary myelofibrosis (PMF). Single-target assays may be employed sequentially but multiplex assays, typically using NGS, sequence several targets in parallel and are more cost effective. Either approach is acceptable if laboratory turnaround times and assay sensitivity (10) are satisfactory (e.g. detection of 1–3% variant allele frequency (VAF) or lower for *JAK2* c.1849G>T (p.Val617Phe), usually referred to as *JAK2* V617F, and 5% VAF for *JAK2* exon 12, *CALR* exon 9 or *MPL* exon 10 variants). The use of broad myeloid NGS panels to screen cases with suspected MPN is unlikely to be cost effective, but if larger panels are used we recommend that the initial analysis and report should be limited to common MPN driver mutations (Table 1).

Universal reporting of mutant allele burden on diagnostic samples is not essential, although this should be considered where prognostically useful, e.g. suspected progression of PV to post-PV myelofibrosis (MF) (11), or where demonstration of molecular response will be relevant (see section 1.3). Low allele burden results (e.g. <1% *JAK2* V617F) should be reported as such, since the clinical significance may be less certain given the prevalence of low level *JAK2* V617F in the general population (see below). In patients with low level *JAK2* V617F and MPN phenotype, screening

101 for *CALR* and *MPL* mutations should be carried out as these mutations may coexist
102 (12). *JAK2* V617F and *CALR* mutations may also coexist with *BCR-ABL1*, with such
103 cases usually being identified following the persistence of thrombocytosis or other
104 MPN features despite achievement of a good molecular response to tyrosine kinase
105 inhibitor therapy for CML (13, 14). Specific *CALR* mutations (type 1, 52bp deletion;
106 type 2, 5bp insertion; type 1-like and type 2-like) (15) have prognostic significance in
107 PMF (Table 2) and should be reported routinely.
108
109 Clinical context must be considered prior to performing screening assays. In patients
110 with erythrocytosis or thrombocytosis, molecular screening investigations (Table 1)
111 are recommended in those with persistently and significantly elevated counts
112 (haematocrit >0.52 l/l in males or >0.48 l/l in females; platelet count ≥450 x 10^9/l) (3,
113 4), after exclusion of secondary causes or where abnormalities are out of keeping
114 with any possible secondary cause. Exclusion of *BCR-ABL1* is important for all
115 patients with thrombocytosis lacking a *JAK2, CALR* or *MPL* mutation or with atypical
116 features (e.g. basophilia, left-shifted granulocytes, small hypolobated
117 megakaryocytes). *JAK2* V617F is also found in healthy individuals, at increasing
118 prevalence with older age ("clonal haematopoiesis", CH) (16-20). Although CH is
119 associated with increased risk of developing cardiovascular disease (21), there is no
120 prospective evidence to guide management of most patients with normal or near-
121 normal blood counts who harbour *JAK2* V617F but do not fulfil diagnostic criteria for
122 MPN, even if there are also abnormalities on bone marrow histology. The *JAK2* 46/1
123 haplotype, and common polymorphisms in *TERT* and other genes only confer a
124 weak predisposition to MPN and therefore there is no clinical value in screening for
125 these in routine practice (22, 23).

126

127 In patients with normal blood counts and atypical thrombosis, molecular screening
128 investigations are recommended where a positive result will inform aetiology and
129 assist management. *JAK2* V617F is particularly associated with splanchnic vein
130 thrombosis, whilst *CALR* mutations are uncommon, especially with normal blood
131 counts. Both mutation types have been detected infrequently in patients with
132 cerebral vein thrombosis without an MPN (*JAK2* more frequently than *CALR*), and in
133 all of these settings there is a lack of evidence-based management guidelines (24-
134 29). In patients with normal blood counts and other atypical sites of thrombosis, there
135 is currently inadequate evidence to recommend molecular screening investigations
136 since the significance of a positive result and consequences for management
137 recommendations are uncertain. However, in patients with arterial or unprovoked
138 venous thrombosis who have a mildly or variably elevated haematocrit or platelet
139 count, not reaching the criteria above, screening may be considered to inform
140 possible aetiology and to prompt close blood count surveillance if cytoreduction is
141 not commenced immediately.

142

143 • **Molecular screening for *JAK2*, *CALR* and *MPL* variants as appropriate**
144 **(Table 1) is recommended in patients with persistent erythrocytosis or**
145 **thrombocytosis (GRADE 1B)**
146 • **Screening for *JAK2* V617F is recommended in cases with normal blood**
147 **counts and unexplained splanchnic vein thrombosis (GRADE 1B) and**
148 **may be considered in selected patients with unexplained cerebral vein**
149 **thrombosis (GRADE 2C)**

150 • **Screening for *CALR* variants may be considered in patients with**
151 **splanchnic vein thrombosis or cerebral vein thrombosis (GRADE 2C)**
152 • **Screening for *JAK2*, *CALR* and *MPL* variants should be considered for**
153 **patients with arterial or unprovoked venous thrombosis who have a**
154 **mildly or variably elevated haematocrit or platelet count that persists**
155 **for 2–3 months (GRADE 2C)**
156 • ***BCR-ABL1* should be excluded in cases with persistent thrombocytosis**
157 **negative for *JAK2, CALR* and *MPL* variants or with atypical features**
158 **(GRADE 1B)**
159
160 **Testing for additional somatic driver variants with myeloid gene small variant**
161 **“panels” +/- cytogenetic analysis**
162 Additional somatic mutations in cancer driver genes include small variants (single
163 nucleotide substitutions or small insertions/deletions) in *TET2* (10-15% MPN),
164 *ASXL1* (5-10%) and *DNMT3A* (5-10%) (30-32), all of which are also associated with
165 CH (16-19). Mutations are found at lower prevalence in regulators of splicing
166 (*SRSF2, SF3B1, U2AF1, ZRSR2*) and of chromatin structure, epigenetic functions
167 and cellular signalling (e.g. *EZH2, IDH1, IDH2, CBL, KRAS, NRAS, STAG2, TP53*)
168 (32). Frequencies are often higher in PMF, post-PV or post-ET MF, and/or blast
169 phase of other MPN or MDS/MPN.
170
171 The improved cost effectiveness of NGS technologies now permits widespread
172 testing for panels of such “myeloid gene” variants which, as a minimum for MPN,
173 should include the genes listed under M85.2 in the NGTD (the current version can be
174 found at https://www.england.nhs.uk/publication/national-genomic-test-directories/).

There is a general consensus that reporting abnormalities down to 5% variant allele frequency (VAF) is adequate for routine analysis, but standardised interpretation of panel results needs further development. For all myeloid neoplasms panel analysis can be performed with DNA extracted from peripheral blood, but DNA extracted from bone marrow is preferred if available. Running and reporting panels is relatively expensive, and in older populations can also identify incidental CH. Use of a panel for all MPN patients is therefore currently neither necessary nor easily deliverable, but panels can add useful supplementary information in specific situations, as detailed below.

Cytogenetic abnormalities are most often found in PMF or post-PV/post-ET MF, in which an abnormal karyotype is reported in up to 45% of patients (33, 34). Conventional karyotyping identifies the commoner copy number abnormalities and deletions (e.g. 20q-, 13q-, +8, +9, 1q+, -7/7q-) and less common balanced translocations (e.g. t(1;6)) (35), and has been incorporated into several prognostic scoring systems (36-38). Other genome-wide technologies such as large pan-cancer NGS panels and SNP (single nucleotide polymorphism) arrays identify the common copy number losses and gains with greater resolution than conventional cytogenetics but will not identify balanced translocations. However these assays may also detect regions of copy-number neutral loss of heterozygosity (LOH) that are not identified by conventional karyotyping but are included in some prognostic models (32). An abnormal karyotype is reported at diagnosis in 5–10% of patients with ET and ~15% with PV (39-41), and although such findings may have some prognostic significance, first line management is not generally altered as a result.

200 **a) At presentation of a suspected MPN, with negative screening investigations**

201 *i) Erythrocytosis.* Patients with unexplained erythrocytosis who lack *JAK2* V617F
202 may be considered for a bone marrow biopsy and *JAK2* exon 12 mutation screening;
203 most are diagnosed with “idiopathic” erythrocytosis if there is no apparent secondary
204 cause (3). The rare entity of *JAK2*-unmutated PV is still recognised in patients with
205 other myeloproliferative clinicopathological features and marrow histology (3) but its
206 molecular aetiology is mostly undefined. A very small number of *JAK2*-unmutated
207 cases with clonal erythrocytosis due to somatic mutations in the *SH2B3* gene have
208 been reported, although the phenotype was of idiopathic erythrocytosis with
209 suppressed erythropoietin rather than classic PV (42) and optimal management of
210 such cases is unknown. There is currently insufficient evidence to recommend
211 myeloid gene panel testing or cytogenetic analysis in the great majority of cases with
212 *JAK2*-unmutated erythrocytosis. Testing may be considered in rare patients with true
213 *JAK2*-unmutated PV, although there is no evidence to guide such practice. Other
214 patients with *JAK2*-unmutated erythrocytosis may be considered for testing for
215 congenital causes of erythrocytosis, as discussed elsewhere (3, 43).

216

217 *ii) Thrombocytosis or suspected PMF.* In the 10-15% of patients with ET and PMF
218 who lack mutations in *JAK2, CALR* or *MPL*, the finding of an additional driver
219 mutation in a myeloid gene panel can support the diagnosis of a clonal disorder, with
220 the proviso that incidental CH could be found in older individuals. The likelihood of
221 identifying a mutation in such patients depends on age, clinical presentation, and
222 gene panel content. More than half of patients with “triple-negative” PMF do harbour
223 additional mutations when screened with comprehensive genomic assays (32) and
224 approximately a third have an abnormal karyotype (35). In patients with bone marrow

225 histology and clinical features consistent with PMF, myeloid gene panel testing in
226 combination with conventional karyotyping (or SNP array) is recommended.
227
228 The diagnosis of triple-negative ET is made on bone marrow histology, although
229 distinction from reactive causes can be challenging, especially in those with mild
230 thrombocytosis. A small minority harbour a non-canonical mutation in *JAK2* or *MPL*,
231 or in another driver gene (32, 44, 45). However in a large analysis of recurrent
232 genomic abnormalities in myeloid neoplasms, no mutations or chromosomal
233 abnormalities were found in over 80% of patients with "triple-negative" ET, including
234 all those aged under 39 years (32). It remains possible that at least a subset of these
235 patients may not have a clonal disorder (46). In older patients there is a higher
236 likelihood of finding an additional driver mutation (or occasionally a chromosomal
237 copy number abnormality or LOH, e.g. chromosome 20); however, the risk of
238 incidental CH also increases. Other differential diagnoses including MDS/MPN
239 should be considered in triple-negative patients with other "myeloid" mutations,
240 through correlation with blood counts and marrow appearances.
241
242 In patients with thrombocytosis who test negative for MPN phenotype driver
243 mutations, there is insufficient evidence to support unselected myeloid gene panel
244 testing. Retesting for MPN phenotype driver mutations appears to be of minimal
245 value but may be considered at occasional (e.g. 5 year) intervals for cases with
246 persistent thrombocytosis. Bone marrow histology remains the key investigation to
247 confirm a diagnosis of MPN in such cases. Moreover in young patients with
248 confirmed low-risk ET, there is no evidence to support cytoreduction (47) and low-
249 dose aspirin therapy has a very limited evidence base (48), meaning that most

patients can be managed expectantly. However myeloid gene panel testing and cytogenetic analysis or other techniques for copy number abnormalities may be considered to look for a clonal marker in some situations:

- Younger patients (e.g. under 60 years) with bone marrow histology typical of ET (or MPN-U or suspected prefibrotic MF) where confirmation of a clonal disorder would be useful in view of the patient's likely long-term disease course and ideally where a broad panel that covers non-canonical variants in *JAK2* and *MPL* and a range of other driver genes is available.
- Patients with significant thrombocytosis (e.g. platelet count >600 × 10^9/l), no reactive cause and borderline bone marrow histology, where cytoreduction would be indicated if there was convincing evidence of a clonal disorder. Examples would include those with an unexplained thrombotic event, particularly younger patients. For older patients without thrombosis, testing may be considered but results must be interpreted with caution in view of the possibility of incidental CH.

Testing is not indicated in patients with normal or reactive bone marrow histology. A myeloid gene panel and cytogenetic analysis is also indicated in patients with bone marrow features suggestive of MDS or MDS/MPN. A summary of genetic testing for suspected MPN that test negative for phenotype driver mutations is shown on Figure 1.

- **A myeloid gene panel and cytogenetic analysis (or equivalent) is recommended for patients with bone marrow histology and clinical features consistent with PMF (+/- suggestive features of MDS or MDS/MPN) who test negative for *JAK2/CALR/MPL* (GRADE 1B).**

275 • **A myeloid gene panel and cytogenetic analysis (or equivalent) is not**
276 **recommended for most patients with *JAK2/CALR/MPL*-negative**
277 **erythrocytosis or thrombocytosis but may be considered in individual**
278 **cases (GRADE 2C).**
279
280 **b) Patients with a known *JAK2, CALR* or *MPL* mutation**
281 In patients with a confirmed clonal disorder, a myeloid gene panel and/or cytogenetic
282 analysis can add information about diagnosis or prognosis at presentation or at
283 suspected transformation; in future it may add information about options for targeted
284 therapy.
285
286 Supplementary molecular information may allow definition of an alternative diagnosis
287 associated with *JAK2* V617F such as MDS/MPN. In patients with a clinical
288 presentation suggestive of an MPN and a *JAK2, CALR* or *MPL* mutation, but with
289 additional cytopenias(s) at diagnosis and unexplained ring sideroblasts or other
290 morphological dysplasia, or with significant peripheral blood monocytosis
291 (monocytes $\geq 1 \times 10^9$/l), myeloid gene panel testing and cytogenetic analysis are
292 recommended. The finding of other driver mutations may either support an
293 alternative diagnosis (e.g. *SF3B1* mutation in MDS/MPN with ring sideroblasts and
294 thrombocytosis) or provide supportive information where the differential diagnosis is
295 challenging (e.g. MPN with monocytosis *vs* CMML).
296
297 For patients with ET and PV who develop cytopenias during cytoreductive therapy,
298 marrow assessment may show morphological dysplasia with a differential diagnosis
299 of disease progression *vs* therapy-related morphological changes. In this context

300 myeloid gene panel testing and cytogenetic analysis may be considered. However,
301 the finding of additional driver mutations is not evidence of disease progression *per*
302 *se* in the absence of baseline molecular information. The number and nature of such
303 variants must be considered in conjunction with a detailed clinical and drug history.
304
305 The presence of additional somatic driver mutations carries prognostic significance
306 in PMF, PV and ET (37, 39, 49). Prognostic scoring models can incorporate
307 molecular information in a variety of ways, from single gene information to
308 comprehensive genomic and cytogenetic / copy number profiles (49). Several high
309 molecular risk (HMR) genes are recognised, (Table 2), but mutations in other genes
310 (e.g. *TP53*) are also important prognostic indicators (50). Myeloid gene panel testing
311 and conventional karyotyping are of most utility, and are recommended, in patients
312 with PMF or post-PV/post-ET MF who are candidates for allogeneic stem cell
313 transplantation in whom decisions can be informed by accurate prognostic
314 information (51). In all other patients, testing may be considered for prognostic
315 purposes if the additional genomic data will guide clinical management, for example:
316 1) Younger patients who at diagnosis fulfil BSH criteria for ET, PV or
317 myeloproliferative neoplasm, unclassifiable (MPN-U) but have atypical clinical
318 features that warrant additional closer surveillance, e.g. marked splenomegaly,
319 atypical bone marrow histology (including those meeting WHO criteria for prefibrotic
320 MF) (1).
321 2) Patients with MPN who are not candidates for allogeneic transplantation but in
322 whom comprehensive prognostic information would aid clinical management and
323 discussion with the patient.
324 3) Patients requiring testing as part of entry to a clinical trial.

325

326 Targeted therapies are now available for patients with acute myeloid leukaemia
327 (AML) who harbour specific driver mutations such as in *IDH1/2* (52, 53). The latter
328 are found in chronic-phase MPNs, but such therapies have not yet been tested in
329 this setting. In patients with blast phase MPNs, myeloid panel testing is
330 recommended for prognostic risk stratification (54-56) or if knowledge of driver
331 mutations could support eligibility for a targeted therapy or entry to a clinical trial.
332 Repeat testing during chronic phase is rarely helpful, although additional mutations
333 may emerge at transformation (31).

334

335 • **Myeloid gene panel testing is recommended for MPN cases who test**
336 **positive for *JAK2/CALR/MPL* mutations and have additional**
337 **cytopenias(s) at diagnosis, unexplained ring sideroblasts or other**
338 **dysplasia, increased blasts (including blastic transformation),**
339 **peripheral blood monocytosis or atypical clinical features (GRADE 1B)**
340 • **Myeloid gene panel testing and conventional karyotyping are**
341 **recommended for all patients with PMF, post-PV or post-ET MF who are**
342 **candidates for allogeneic stem cell transplant (GRADE 1B)**
343 • **Myeloid gene panel testing should be considered for other patients if**
344 **the additional genomic data will guide clinical management (GRADE**
345 **2C)**

346

347

348 **Disease monitoring: quantitative assays of clonal burden**

349 Quantitative assays of *JAK2* V617F mutant allele burden have been employed to
350 assess clonal dynamics in clinical trials, with molecular responses being reported
351 with drugs including pegylated interferon-alfa and ruxolitinib (57-62). A small number
352 of patients taking pegylated interferon-alfa were reported to maintain complete
353 molecular remissions for over a year off therapy (58). These studies have not yet
354 confirmed that achieving a particular level of molecular response is associated with
355 more favourable vascular or transformation risk and molecular response is therefore
356 not currently considered a formal treatment target. At present there is therefore no
357 evidence to recommend routine quantitative monitoring of clonal burden. However
358 assessment using quantitative, high-sensitivity assays (e.g. real time quantitative
359 PCR or digital PCR) of mutant allele burden may be considered, e.g. on an annual
360 basis, in patients who are in haematological response on low-dose pegylated
361 interferon alfa, where a confirmed molecular remission would support a further dose
362 reduction or trial without therapy. These assays can also be used in patients
363 following post-allogeneic stem cell transplant to monitor for residual disease and
364 guide early intervention with donor-lymphocyte infusion prior to clinical relapse (63,
365 64). Fluorescence *in situ* hybridisation (FISH) for any known cytogenetic
366 abnormalities can also be helpful to monitor disease following therapy, albeit with
367 more limited sensitivity than most quantitative PCR assays.
368
369 • **High-sensitivity assays of mutant allele burden are recommended**
370 **following post-allogeneic stem cell transplant to monitor for residual**
371 **disease (GRADE 1C).**

372 • **Quantitative assays of mutant allele burden are not recommended for**
373 **most MPN patients but may be considered where demonstration of**
374 **molecular response would influence clinical management (GRADE 2C).**
375
376 **Atypical myeloproliferative neoplasms**
377
378 **CEL and MLN-eo**
379 Patients with persistent eosinophilia of at least 1.5 × 10^9/l with no obvious secondary
380 cause should be investigated for *FIP1L1-PDGFRA* on peripheral blood or bone
381 marrow by FISH or nested reverse transcriptase polymerase chain reaction (nested
382 RT-PCR) (7, 65). Either technique alone may miss occasional cases (66, 67) and so
383 both, or other supplementary approaches (66, 68), should be considered in cases
384 with a high index of suspicion.
385
386 Almost all tyrosine kinase (TK) gene fusions apart from *FIP1L1-PDGFRA* are
387 associated with visible cytogenetic rearrangements and therefore bone marrow (BM)
388 cytogenetic analysis should ideally be performed for cases with a suspected myeloid
389 neoplasm if *FIP1L1-PDGFRA* is not detected (7, 65). The diversity of fusions
390 precludes effective targeted RT-PCR analysis, although an increasing number of
391 cases are being picked up by broad or targeted RNAseq screens. Although effective,
392 this approach is currently too expensive to recommend as a general screening tool in
393 all but exceptional cases. Break-apart FISH analysis for specific loci (*PDGFRA*,
394 *PDGFRB*, *FGFR1*, *JAK2* for MLN-eo; *ABL1*, *FLT3*, *ETV6*, other TK genes for CEL)
395 may also be used to identify disruption of key loci. It is important that any suspected
396 fusion (including *FIP1L1-PDGFRA*) identified by cytogenetics or FISH is confirmed

397 by molecular methods to ensure that targeted therapy is used appropriately and to
398 facilitate subsequent molecular monitoring, which is available for *FIP1L1-PDGFRA*
399 and most other fusions in specialist centres. The timing of tests should follow that
400 recommended for CML, including more frequent tests for patients attempting
401 treatment-free remission (2, 69, 70).
402 Mastocytosis should be considered if serum tryptase is elevated in the absence of a
403 TK gene fusion and examination of bone marrow histology is essential in this
404 context. If negative for the markers above, a myeloid panel or targeted analysis
405 should be considered to detect other markers of clonality associated with
406 eosinophilia (Table 3) (71-75).
407

408 • **Patients with persistent eosinophilia should be investigated initially for**
409 ***FIP1L1-PDGFRA* by FISH and/or nested RT-PCR (GRADE 1B).**
410 • **Bone marrow cytogenetics or FISH is recommended to screen for other**
411 **fusion genes, which must then be confirmed by molecular methods**
412 **(GRADE 1B).**
413 • **Myeloid gene panel and *KIT* D816V testing should be considered for**
414 **patients with persistent unexplained eosinophilia who test negative for**
415 **fusion genes (GRADE 2B).**
416

417 **CNL, MPN-U**

418 *CSF3R* mutations are strongly, but not exclusively, associated with chronic
419 neutrophilic leukaemia (CNL (76, 77) and are a central diagnostic feature of this
420 disorder (1). Wider genomic profiling indicates a significant overlap in the pattern of
421 mutated genes between CNL and MDS/MPN (78) suggestive of a disease

422 continuum. *ASXL1* mutations were associated with an adverse prognosis in CNL in
423 one study (79), but did not influence response to ruxolitinib (80).
424
425 MPN-U is an uncommon subtype consisting largely of cases that fail to meet the
426 diagnostic criteria for a specific MPN subtype, or present with features that overlap
427 with two or more subtypes. As such, most cases test positive for *JAK2* V617F, *CALR*
428 or other myeloid driver mutations (81).
429
430 - **Testing for *CSF3R* variants, preferably as part of wider myeloid panel,**
431 **is recommended for all patients with suspected CNL (Grade 2B)**
432
433 **Mastocytosis**
434 Up to 90% of adult systemic mastocytosis (SM) cases across all subtypes test
435 positive for *KIT* c.2447A>T; p.(Asp816Val), usually referred to as *KIT* D816V. Due to
436 the nature of the disease the VAF is often too low for detection by NGS and thus
437 targeted, sensitive methods such as real time quantitative PCR or digital PCR are
438 often required for analysis of peripheral blood or bone marrow samples.
439 Alternatively, standard mutation analysis may be performed on purified mast cells
440 (82, 83). In many cases *KIT* D816V is detectable in peripheral blood (84) but, if
441 negative, analysis of a BM sample should be considered if there is a high index of
442 suspicion. If *KIT* D816V is not detected in the marrow and there is a strong clinical
443 suspicion of mastocytosis, a wider screen for D816 variants or other *KIT* mutations
444 should be considered. In children with mastocytosis, *KIT* D816V is only seen in 30–
445 50% of cases and other activating *KIT* mutations account for most of the remaining
446 cases (82, 85).

- **Sensitive testing for *KIT* D816V is recommended for all patients with a clinical suspicion of mastocytosis (GRADE 1B).**
- **If negative for *KIT* D816V, screening for other *KIT* mutations should be considered for adults (but is recommended for children) (GRADE 1B).**

Additional somatic mutations are found in 70–90% of advanced SM patients. Most mutation-positive cases have SM with an associated haematological neoplasm (SM-AHN), with the AHN usually being a subtype of MDS/MPN. Mutations are less frequent (<20%) in patients with indolent SM (ISM) (86, 87). In advanced SM, mutations in *SRSF2*, *ASXL1*, *RUNX1*, *EZH2* and *NRAS* have been associated with an adverse prognosis and thus molecular profiling is useful to guide transplant decisions (83, 87-89). In ISM, high VAF (≥30%) mutations in *ASXL1*, *RUNX1* and/or *DNMT3A* have been associated with an adverse prognosis (87) but the value of routine molecular profiling in this subtype remains to be established.

An abnormal karyotype is seen in a quarter of SM-AHN cases but is infrequent in other subtypes. An abnormal karyotype, and particularly a poor-risk karyotype (e.g. monosomy 7, complex karyotype) is associated with an adverse prognosis but there is disagreement as to whether or not this effect is independent of mutational status (90, 91).

- **Myeloid panel analysis is recommended for patients with advanced SM who are candidates for allogeneic stem cell transplantation (GRADE 1B).**

472 • **Myeloid panel analysis may be considered for other SM patients if the**
473 **apparent aggressiveness of the disease might influence options for**
474 **therapy (GRADE 2B).**
475 • **Myeloid panel and/or bone marrow cytogenetics should be considered**
476 **to characterise the AHN component of SM-AHN (GRADE 2B)**
477
478 **Myelodysplastic/myeloproliferative neoplasms**
479 The diagnosis of the adult MDS/MPN overlap syndromes - chronic myelomonocytic
480 leukaemia (CMML), atypical CML *BCR-ABL1*-negative (aCML), MDS/MPN with ring
481 sideroblasts and thrombocytosis (MDS/MPN-RS-T) and MDS/MPN-unclassifiable
482 (MDS/MPN-U) – remain heavily reliant on bone marrow morphology and clinical
483 assessment. Molecular genetics can however provide key information to assist with
484 diagnosis, sub-classification and prognostication across the spectrum of these
485 disorders.
486
487 **Initial investigations in suspected MDS/MPN**
488 In patients with a suspected MDS/MPN it is essential to exclude *BCR-ABL1* in all
489 cases and also exclude rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1* or *PCM1-*
490 *JAK2* in the rare cases with an associated eosinophilia (1). Cytogenetics should also
491 be performed at the time of a confirmed diagnosis (8) due to its importance for both
492 demonstrating clonality and informing prognosis. This should also exclude any rare
493 TK gene fusions which have been reported to mimic MDS/MPN (92). Cytogenetic
494 abnormalities can be identified in 30–50% of cases using conventional karyotyping
495 with the most common abnormalities being +8, +9, -7, del7q, del20q, del13q and
496 isochromosome 17q (93, 94). The detection of +8, abnormalities of chromosome 7

497 or a complex karyotype have been reported as poor risk in CMML (95). In the
498 absence of a cytogenetic sample or failed result, single nucleotide polymorphism
499 array (SNP-array) analysis should be considered which could increase the yield of
500 detecting an abnormality to 75% (96). FISH, in particular for chromosomes 7 and 8,
501 should be performed as a minimum requirement (8).
502
503 • ***BCR-ABL1* should be excluded in all cases of suspected MDS/MPN,**
504 **and rearrangements associated with MLN-eo should be excluded in**
505 **cases with eosinophilia (GRADE 1B).**
506
507 **Testing for additional somatic driver mutations with myeloid gene "panels"**
508 *(i) In patients with indeterminate morphology*
509 Somatic mutations are consistently reported to occur in >90% of cases across the
510 MDS/MPN overlaps (97-100). The high frequency of somatic mutations in these
511 conditions means the presence of a mutation can provide supportive evidence of
512 clonality and assist in difficult diagnostic scenarios. Concerns have however been
513 raised regarding the use of mutational analysis in this setting, due to reports of
514 frequent somatic mutations in aging healthy individuals (16-19). A recent study
515 however, in patients investigated for possible CMML, confirmed that even in the
516 absence of definitive morphological features, those patients with a somatic mutation
517 had a clinical phenotype and genotype indistinguishable from those with disease,
518 and comparably poor outcomes (101). A myeloid gene panel is therefore
519 recommended in difficult diagnostic cases and the presence of two mutations, one of
520 which has a high VAF (>20%) would support a diagnosis (8). The genes included in
521 the current NGTD for suspected MDS/MPN overlaps are listed in Table 4 (see also

522 NGTD test code M224.1) and the minimum genes recommended for the
523 investigation of patients with suspected CMML in Table 5. It is accepted that the
524 genes included in the NGTD panel are a minimum requirement and larger panels
525 may provide additional information, e.g. abnormalities of *NPM1* are uncommon in
526 MDS/MPN but identify cases likely to transform rapidly to AML, whereas
527 abnormalities of *FLT3* are potential therapeutic targets (8).
528
529 *(ii) In patients with a confirmed diagnosis of MDS/MPN.* The genes most commonly
530 mutated in MDS/MPN are not specific for these conditions; however
531 genotypic/phenotypic correlations have been identified which can assist in sub-
532 classification. Mutations in genes with prognostic relevance can also be identified
533 along with possible targets for therapy (*JAK2, IDH1/2*) with the latter likely to
534 increase over time.
535
536 With respect to CMML, *SRSF2, TET2* and *ASXL1* are by far the most commonly
537 mutated genes (97-99) and the combination of mutation in *TET2* and either *SRSF2*
538 or *ZRSR2* is highly specific for a myelomonocytic phenotype (102). A diagnosis of
539 aCML is supported by the presence of mutations in *SETBP1* and/or *ETNK1* which
540 are reported in ~25–38% and ~10% of cases respectively (103-106). These genes
541 are mutated less frequently in CMML and MDS/MPN-U although *SETBP1* is also
542 mutated in CNL (103, 105). Patients with aCML also show a relative lack of MPN-
543 phenotype driver mutations (*JAK2*, *CALR, MPL*) (93, 107) with the presence of these
544 tending to exclude this diagnosis (1). In MDS/MPN-RS-T, mutations in *SF3B1* and
545 *JAK2* are reported in up to 90% and 57% of cases, with *CALR* or *MPL* mutations in a
546 small minority (108-110) and the detection of an *SF3B1* mutation in patients with

547 15% ring sideroblasts can help define the diagnosis (1). Co-mutation of these genes
548 would strongly support a diagnosis of MDS/MPN-RS-T though is not a current
549 requirement (1). Elevated tryptase and/or mast cell abnormalities in MDS/MPN
550 suggests SM-AHN, which is often underdiagnosed but may be supported by the
551 finding of *KIT* D816V (111). The detection of *KIT* D816V in the context of a confirmed
552 MDS/MPN should trigger review of bone marrow morphology for a possible co-
553 existing mastocytosis.
554
555 Mutational analysis is now incorporated into prognostic scoring systems across
556 these diseases. Four genes (*ASXL1, NRAS, RUNX1 and SETBP1*) are
557 independently associated with a worse overall survival (OS) in CMML and have been
558 incorporated into the most recent CMML-specific prognostic scoring system (CPSS)-
559 molecular and analysis of these is defined as mandatory for risk assessment (8).
560 The number of mutations per patient has also been shown to correlate inversely with
561 OS (97), and *ASXL1* and/or *NRAS* mutations are associated with worse survival
562 after stem cell transplantation (112). *ASXL1* and *SETBP1* also infer a poor
563 prognosis across other MDS/MPN with these genes being commonly co-mutated
564 (100, 103, 105, 113). In atypical CML, *SETBP1* was associated with an adverse
565 clinical phenotype and a significantly worse OS (103, 105) while both *SETBP1* and
566 *ASXL1* were associated with poor survival in patients with MDS/MPN-RS-T and have
567 been incorporated into a mutation enhanced prognostic model (113).
568
569 A targeted sequencing panel is therefore recommended in patients diagnosed with
570 an MDS/MPN overlap disorder, particularly those being considered for active
571 treatment or allogeneic transplantation (8, 94). Mutational analysis can also provide

572 prognostic information and potentially identify therapeutic targets in patients not
573 eligible for intensive treatment and analysis may therefore be considered even in
574 those receiving supportive care (8, 94). There is strong concordance between
575 mutations detected in the peripheral blood and bone marrow, particularly in CMML,
576 and mutational analysis has both a high positive and a high negative predictive value
577 for a subsequent diagnosis (101). Mutational analysis is therefore a potential option
578 in elderly patients or those unfit for a bone marrow biopsy to either confirm the
579 presence of a clonal marker or eliminate the need for invasive testing.
580

581 • **Myeloid gene panel analysis and bone marrow cytogenetics or SNP**
582 **array is recommended for patients diagnosed with MDS/MPN and for**
583 **cases with suspected MDS/MPN but with indeterminate morphology**
584 **(GRADE 1B).**
585

586 **Future directions**

587 The landscape of genetic and genomic testing is changing rapidly, with broad
588 screening techniques such as large pan-cancer panels, whole genome sequencing
589 and RNAseq beginning to impact on routine practice. Genomic, transcriptomic and
590 epigenetic profiling of single cells are providing novel insights into the complexity and
591 diversity of clonal disorders. Whilst these approaches clearly have huge potential,
592 e.g. in chronic myeloid neoplasms, they will facilitate comprehensive prognostic
593 modelling (49), detection of rare targetable gene fusions (114) and potentially cell
594 type-specific assessment of measurable residual disease (115), it is currently
595 unclear when or whether they will be cost effective compared to more diverse,
596 targeted approaches.

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Review Process

Members of the writing group will inform the writing group Chair if any new pertinent evidence becomes available that would alter the strength of the recommendations made in this document or render it obsolete. The document will be archived and removed from the BSH current guidelines website if it becomes obsolete. If new recommendations are made an addendum will be published on the BSH guidelines website (www.b-s-h.org.uk).

622

623 **Disclaimer**

624 While the advice and information in this guidance is believed to be true and accurate
625 at the time of going to press, neither the authors, the BSH nor the publishers accept
626 any legal responsibility for the content of this guidance.

627

628 **Figure legend**

629 Figure 1. Summary of genetic testing for suspected MPN that test negative for
630 common MPN phenotype driver mutations

631
632 **Table 1. Peripheral blood screening targets in suspected MPN**
633

Presentation	Variant	Frequency	Reference
Erythrocytosis	*JAK2* V617F	96–97% PV	(116, 117)
	JAK2 exon 12 mutations*	~3% PV	(117)
Thrombocytosis	*JAK2* V617F	50–60% ET	(118, 119)
	CALR exon 9 mutation	25–30% ET	(30, 120)
	MPL exon 10 mutation	3–11% ET	(121, 122)
	BCR-ABL1 fusion	To exclude CML	
Suspected primary myelofibrosis	*JAK2* V617F	50–60% PMF	(120, 123)
	CALR exon 9 mutation	15–35% PMF	(30, 120)
	MPL exon 10 mutation	6–9% PMF	(120, 122)
Suspected chronic myeloid leukaemia	*BCR-ABL1* fusion	100% CML	

634
635
636 * Rare cases with a discrepancy between *JAK2* exon 12 mutant allele burden in bone marrow and
637 peripheral blood have been reported, so testing of bone marrow may be considered if there is a high
638 index of suspicion (124).
639
640
641
642

643 **Table 2. Prognostic scoring systems for PMF, post-PV and/or post-ET MF**
644 **incorporating cytogenetic and/or molecular information**
645

Score	Disorder	Cytogenetic / molecular variable(s) included	HR	Reference
DIPSS+	PMF	Unfavourable karyotype*	2.4	(36)
MYSEC-PM	Post-PV/post-ET MF	*CALR*-unmutated	2.6	(125)
MIPSS70	PMF	Absence of *CALR* type 1/type 1-like mutation At least 1 HMR† mutation 2 or more HMR† mutations	1.89 1.77 3.95	(126)
MIPSS70+	PMF	Absence of *CALR* type 1/type 1-like mutation At least 1 HMR† mutation 2 or more HMR† mutations Unfavourable karyotype**	2.4 1.8 2.4 3.1	(126)
GIPSS	PMF	Very high risk karyotype*** Unfavourable karyotype*** Absence of *CALR* type 1/type 1-like mutation *ASXL1* mutation *SRSF2* mutation *U2AF1* mutation	3.1 2.1 2.1 1.8 2.4 2.4	(38)
MIPSS70+ v2	PMF	Very high risk karyotype*** Unfavourable karyotype*** 2 or more HMR mutations‡ 1 HMR mutation‡ Absence of type 1/type 1-like *CALR* mutation	5.9 2.5 2.6 1.8 2.1	(37)
Sanger multistage model	MPN	Up to 53 genomic features (single gene variant / copy number information)		(32)
MTSS	PMF/post-PV/post-ET MF	Absence of *CALR* or *MPL* mutation *ASXL1* mutation	2.4 1.42	(127)
FIM	PMF/post-PV/post-ET MF	*TP53* High risk mutations**** *ASXL1* only	8.68 3.24 2.45	(128)

646
647 † HMR = high molecular risk (*ASXL1, IDH1/2, EZH2, SRSF2*)

648 ‡ HMR = high molecular risk (*ASXL1, IDH1/2, EZH2, SRSF2, U2AF1* Q157)
649
650 *Unfavourable: Complex karyotype or sole or two abnormalities that include +8, −7/7q-, i(17q), −5/5q-,
651 12p-, inv(3) or 11q23 rearrangement.
652 **Unfavourable: Any abnormal karyotype other than sole abnormalities of 20q-, 13q-, +9,
653 chromosome 1 translocation/duplication, -Y or sex chromosome abnormality other than –Y
654 ***Very high risk: single/multiple abnormalities of -7, i(17q), inv(3)/3q21, 12p-/12p11.2, 11q-/11q23, or
655 other autosomal trisomies not including +8/ +9 (e.g., +21, +19); Favourable: normal karyotype or sole
656 abnormalities of 13q-, +9, 20q-, chromosome 1 translocation/duplication or sex chromosome
657 abnormality including -Y; Unfavourable: all other abnormalities.
658 **** ≥1 mutation in *EZH2*, *CBL*, *U2AF1*, *SRSF2*, *IDH1*, *IDH2*, *NRAS* or *KRAS*. *ASXL1*-only mutations
659 had no or limited prognostic value, however *ASXL1* mutations conferred a worse prognosis when
660 associated with a mutation in *TP53* or high-risk genes.
661
662
663

Table 3. Molecular abnormalities associated with eosinophilia

Category	Genes	Frequency	Reference
MLN-eo	*FIP1L1-PDGFRA*	5–20% HE_{US}; >80% MLN-eo	(71, 129)
	Other PDGFRA fusions	rare	
	PDGFRB fusions	<10% MLN-eo	
	FGFR1 fusions	<5% MLN-eo	
	PCM1-JAK2, BCR-JAK2	<5% MLN-eo	
Tyrosine kinase gene fusions in CEL and eosinophilia associated with other MPN or MDS/MPN	*ETV6-ABL1*	?1–2% HE_{US}/MPN-eo	
	FLT3 fusions	rare	
	Other *JAK2* fusions	rare	
	NTRK3, *RET*, *ALK*, others	very rare	
Other variants in CEL and eosinophilia associated with other MPN, MDS/MPN or SM	*JAK2* V617F	4% HE_{US}	(71)
	JAK2 exon 13 indels	1–2% HE_{US}	(75, 130)
	KIT D816V	3% HE_{US}	(71)
	STAT5B N642H	2% persistent eosinophilia including MPN-eo and MDS/MPN-eo	(74)
	DNMT3A, TET2, *ASXL1*, *EZH2*, *SETBP1*, *CBL* other myeloid genes	11-21% HES/HE_{US}	(72, 73, 130)

HE_{US}, hypereosinophilia of undermined significance; HES, idiopathic hypereosinophilic syndrome

Table 4. Common abnormalities in CNL and MDS/MPN

Pathway	Gene	aCML	CNL	CMML	MDS/MPN-RS-T	MDS/MPN-U
Signalling	*KRAS*	3-10%	**-**	7-23%	-	4-5%
	NRAS	10-30%	10%	4-38%	-	8-12%
	JAK2	4-11%	8%	1-11%	37-78%	8-25%
	CBL	8-15%	5%	8-23%	3%	7-8%
	KIT	6%	**-**	0-3%	-	4%
	FLT3	5-7%	**-**	1-4%	-	3-4%
	CSF3R	0-25%	60-80%	2-4%	-	4-6%
	SETBP1	7-38%	14-56%	4-18%	1-6%	13-16%
	SH2B3	0-4%	**-**	0-5%		3%
	MPL	-	**-**	rare	4-20%	8%
	CALR	-	**-**	rare	17%	4%
	ETNK1	3-9%	3%	3-4%	**3%**	4%
	PTPN11	0-8%	~0%	3-5%	**-**	4-5%
	NF1	0-4%	**-**	6-10%	**-**	4%
Splicing	*SF3B1*	0-6%	3%	3-10%	97%	11-16%
	SRSF2	37-48%	44%	24-55%	4%	24-48%
	U2AF1	3-15%	15%	2-24%	-	8-19%
	ZRSR2	3-4%	3%	3-8%	-	0-6%
Transcription	*RUNX1*	6-20%	**3%**	8-28%	1%	4-17%
	CEBPA	4%	**-**	0-20%	-	4-8%
	GATA2	15-18%	13%	1-14%	3%	12-16%
	NPM1	4%	**-**	1-3%	-	0-3%
	BCOR	4%	**-**	3-7%	**-**	-
	CUX1	10-11%	**5%**	0-6%	4%	0-8%
	TP53	3%	**3%**	0-2%	3%	0-14%
Cohesin	*STAG2*	11-15%	3%	3%	-	8-16%
DNA methylation	*DNMT3A*	4-7%	5%	2-12%	18%	0-13%
	TET2	16-37%	21%	29-73%	21%	30-44%
	IDH1/2	0-3%	3%	1-7%	3%	0-10%
Histone modification	*ASXL1*	28-92%	57-77%	32-69%	0-11%	53-64%
	EZH2	13-33%	21%	5-13%	7%	10-25%

Data from (8, 77-79, 93, 100, 101, 104, 106, 110) and references therein. A dash indicates a mutation in that gene is rare or has not been reported. The NGTD also includes *CHEK2*, *NFE2, IKZF1* and *HRAS* but the prevalence of mutations in these genes is unknown for CNL and MDS/MPN.

Table 5. Recommended* minimal panel for targeted sequencing in CMML

TET2	*IDH2*	*NF1*	*FLT3*
ASXL1	*BCOR*	*JAK2*	*SRSF2*
DNMT3A	*CBL*	*RUNX1*	*SF3B1*
EZH2	*KRAS*	*SETBP1*	*U2AF1*
IDH1	*NRAS*	*NPM1*	*ZRSR2*

* see (8)

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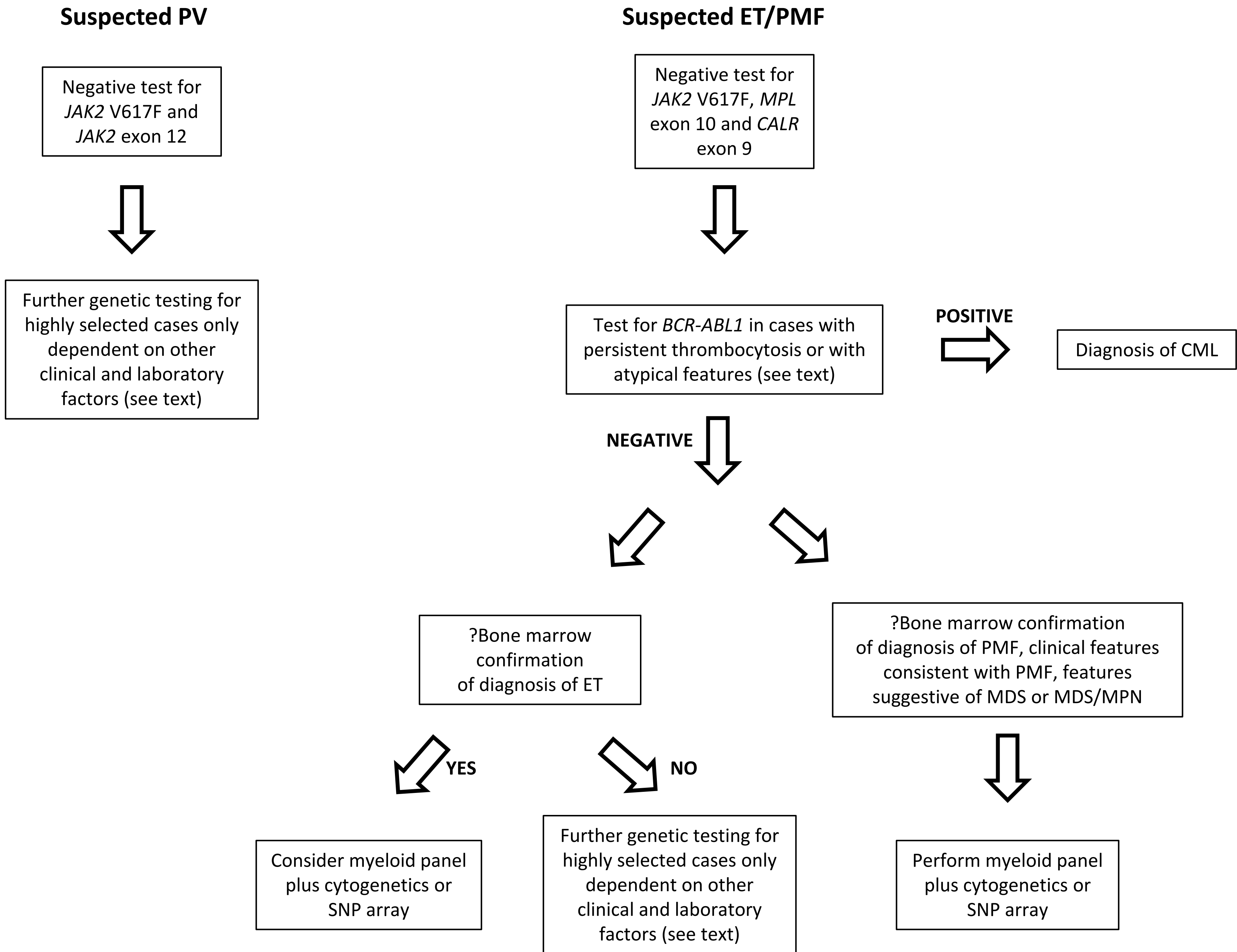

Figure 1