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Age-specific false positives and the clinical pathway for a whole-blood DNA-methylation burden screen in older adults and carriers of clonal hematopoiesis: an open-data evaluation

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Abstract

Background. Whole-blood DNA-methylation readouts are proposed for early detection of myeloid neoplasms, but false positives in their intended users (older adults, many with clonal hematopoiesis of indeterminate potential [CHIP]) are unstudied.

Objective. To measure age-specific false positives of a fixed 48-locus methylation burden call and model what a positive result should trigger.

Methods. Pre-specified, public-data analysis. A positive call was ≥4 of 40 evaluable loci shifted ≥0.20 β from a reference built on 732 adults. It was applied to 917 samples from three cohorts without known myeloid disease (one pipeline from raw arrays), 540 repeat-sampling intervals and, as a sensitivity analysis, 2,639 processed samples. CHIP effects came from a meta-analysis (N = 8,196). A decision model compared single and repeat-draw strategies in CHIP carriers and unselected adults ≥60.

Results. Positive calls rose with age: 0.3% (<60 y), 1.9% (60–69), 2.5% (70–79) and 7.5% (≥80; 95% CI 4.3–12.7). Odds ratio per 10 years 2.22 (1.21–4.07). On repeat sampling, 6 of 17 positive draws (35%; 17–59) remained positive after a median 5.6 years. Published CHIP effects do not reach the call threshold at any locus. At an assumed sensitivity of 0.50 and age 70–79, positive predictive value was 1.8% in unselected adults and 9–29% in CHIP carriers (54% with a repeat draw); net benefit was near zero in unselected adults (at most 0.3 per 1,000) and positive in CHIP carriers (up to 9.8 per 1,000).

Conclusions. False positives are infrequent below 80 years and rise thereafter. The plausible use is in known CHIP carriers: a confirmatory repeat draw, then hematologic work-up. Pre-diagnostic sensitivity remains unmeasured.

Key Points

- A blood methylation test of this kind gives more false alarms as people age, especially after 80, and about a third of false alarms recur on a second test.
- Its use is most promising in people already known to carry clonal blood-cell mutations, where a positive result would prompt a specialist blood work-up after a repeat test.
- How well the test detects disease before diagnosis has not yet been measured.

1 Introduction

Myeloid neoplasms are diagnosed predominantly in older adults; age-specific incidence of acute myeloid leukemia (AML) in the United States rises from 7.9 per 100,000 at 60–64 years to 28.5 per 100,000 at 80–84 years[1]. The same age range carries the highest prevalence of clonal hematopoiesis: somatic mutations in genes recurrently mutated in hematologic cancers are found in 5.6% of persons aged 60–69, 9.5% aged 70–79 and

18.4% aged 90 or older[2], and confer a hazard ratio of 11.1 (95% CI 3.9–32.6) for subsequent hematologic cancer, an absolute risk of approximately 0.5% per year overall and 1% per year when the variant allele fraction (VAF) is greater than 0.10[2]. Most carriers never progress, and CHIP is defined by the absence of cytopenia and dysplasia[3].

DNA-methylation changes in peripheral blood have been reported years before a diagnosis of AML or myelodysplastic syndrome (MDS)[4], which motivates methylation-based early-detection tests. Two features of the intended-use population are a problem for any such test. First, age itself changes blood methylation at a large fraction of CpGs[5], so a reference derived from younger adults may misclassify older ones. Second, CHIP mutations in DNMT3A and TET2 alter methylation in opposite directions[6], so CHIP carriers without neoplasia may test positive. Neither the false-positive rate in older adults nor its persistence over time has been reported for a fixed methylation burden call.

Version 1 of this preprint described a static whole-blood burden score across 48 loci[7]. Here we replace its estimates with a pre-specified evaluation built only on public data and organized around one clinical question: what should a positive result trigger, and how often would that action follow a false positive? We consider two populations side by side—known CHIP carriers, in whom a positive result would prompt hematologic work-up, and unselected adults aged 60 years or older, in whom it would prompt a reflex myeloid mutation panel.

2 Methods

2.1 Design and pre-specification

The statistical analysis plan (SAP) was locked on 8 November 2026 before any test-cohort score was computed. LSADT and GSE55763 had been examined with this panel in earlier work (burden drift and probe coverage)[10], but not with the positive-call rule. The lock fixed the panel, reference, positive-call rule, missing-data rule, pipeline, and excluded cohorts. Nine changes made after results were seen are reported in Supplementary Table S2 and labeled post hoc where they apply. Reporting follows STROBE, with STARD 2015 items where applicable[8,9]. All data are public and de-identified; no ethics approval was required.

2.2 Panel

The 48-locus burden panel (27 genes; 38 loci expected to gain and 10 to lose methylation in disease) is fixed and was published previously[10]; probe identifiers, GRCh38 positions and CpG-island context are listed in Supplementary Table S1. No locus was added, removed or reweighted for this study.

2.3 Reference and positive call

The reference was built from raw HumanMethylation450 IDATs of 732 adults (GSE87571[5]) processed with SeSAMe 1.28 (QCDPB; pOOBAH masking)[11]. For each locus the reference value was the median β and its spread the SD. Loci masked in more than 10% of reference arrays were declared non-evaluable; this rule left 40 evaluable loci and excluded 8 (Supplementary Table S1). A sample was called positive when at least 4 of the 40 loci were shifted by at least 0.20 β from the reference median in the panel's disease direction. A masked cell counted as not shifted; arrays with more than 4 masked loci were excluded and counted. A secondary rule (≥4 loci beyond 2 SD) was reported but not used for any decision. Counts were computed with the study's scoring code and reproduced exactly, on every array, by an independent standalone script deposited with this paper (see Data availability).

2.4 Cohorts

Three cohorts without known myeloid disease were processed from raw IDATs through the same pipeline (Table 1): controls from an epigenome-wide study of rheumatoid arthritis (GSE42861; n = 335)[12]; the Swedish Adoption/Twin Study of Aging (SATSA; E-MTAB-7309; 1,071 arrays from 444 individuals)[13]; and the Longitudinal Study of Aging Danish Twins (LSADT; GSE73115; 86 individuals sampled in 1997 and 2007)[14]. Baseline was the first draw per individual. GEO records for LSADT carry no age; the 1997 wave (published intake age 73–82[14]) was assigned to 70–79 years and the 2007 wave to ≥80 years. For four LSADT individuals with duplicate arrays per

wave, replicate "-1" was used and the pairs served as technical replicates. A fourth cohort (GSE55763; 2,711 arrays)[15] has no released IDATs; its authors' normalized β values and detection P values (masking at P > 0.01) were scored as a separate, labeled sensitivity analysis and not pooled. Cohorts used to derive the panel and the reference cohort were excluded as test sets.

2.5 Analyses

A1, age-specific positive rate. Proportion positive by age band with Wilson 95% CIs, per cohort and pooled. The age effect was estimated by a generalized estimating equation (logit link, cohort term, robust SEs clustered by twin pair) on baseline samples from GSE42861 and SATSA. Leukocyte composition (EpiDISH[16]) could be adjusted only for the secondary rule in GSE42861; for the primary call, it remains an unadjusted competing explanation.

A2, persistence. In SATSA, consecutive draws from the same individual formed intervals; we estimated P(positive at t2 | positive at t1), P(positive at t2 | negative at t1) and new positives per 100 person-years. LSADT provided one 10-year interval per individual.

A3, expected CHIP contribution. Carrier minus non-carrier β differences at the 48 loci were taken from a published meta-analysis of four US cohorts (N = 8,196)[6], signed toward the panel direction and tested at P < 0.05/48. The largest per-locus shift expected for a carrier was obtained by scaling the carrier-average difference to a 60% clone, assuming a carrier-average clone fraction of 0.2.

A4, decision model. For each age band, positive predictive value (PPV), negative predictive value and net benefit[17] were computed for one-step (act on the first positive) and two-step (act only if a repeat draw is also positive) strategies. Inputs were the A1 false-positive rates and the A2 repeat-positive probability among false positives (6/17; 0.75 as a short-interval sensitivity value). Sensitivity was a scenario parameter (0.25, 0.50, 0.75) because no public pre-diagnostic whole-blood array cohort exists. Prevalence of preclinical myeloid neoplasm was the annual rate multiplied by the duration of the undiagnosed phase: in unselected adults, SEER AML incidence[1] plus MDS incidence[18] (log-linear interpolation between the published 65–69 and ≥85 rates) times 1, 2 or 5 years; in CHIP carriers, 0.5–1% per year[2] times 1–2 years. Uncertainty was propagated by 10,000 draws from Jeffreys beta distributions for the observed counts.

A6, pre-diagnostic data (exploratory). Panel CpGs were mapped (hg19) to the regions released for a nested case–control study with blood collected a median of 9.9 years before AML/MDS diagnosis (GSE253126)[4].

3 Results

3.1 Positive calls rise with age

Across 831 baseline samples, 16 were positive (1.9%; 95% CI 1.2–3.1). By age band (baseline samples plus the LSADT 2007 wave; 917 samples), the rate rose from 1/308 (0.3%) below 60 years to 4/208 (1.9%) at 60–69, 6/241 (2.5%) at 70–79 and 12/160 (7.5%; 4.3–12.7) at 80 years or older (Table 2; Fig. 1). The pooled odds ratio per 10 years of age was 2.22 (1.21–4.07; p = 0.010; 745 subjects, 15 events). In SATSA baseline samples alone it was 1.87 (1.00–3.50; p = 0.049); using all 951 SATSA arrays, 2.71 (1.80–4.09). Exclusions for masking were 1/335 arrays in GSE42861, 120/1,071 in SATSA and 0/180 in LSADT; 33 SATSA individuals had no array passing quality control, leaving 411 at baseline. Removing five loci masked more often in SATSA than in the reference (post hoc) gave 3.8% (2.4–6.0) in SATSA.

The secondary 2-SD rule was positive in 65/334 (19.5%) GSE42861 controls and in 214/411 (52.1%; 47.2–56.9) SATSA baseline samples, and is unsuitable as a screening call in this age range.

In the GSE55763 sensitivity analysis (n = 2,639, aged 24–75), rates were 0.05% (<60), 0.5% (60–69; 0.1–1.7) and 1.7% (70–79; 0.5–5.8), with an odds ratio per 10 years of 6.42 (1.76–23.48). Medians at individual loci differed from the reference by −0.05 to +0.12 β in the disease direction, a difference attributable to normalization; these estimates were therefore not pooled.

3.2 One in three positives persists

In SATSA, 540 intervals from 268 individuals (median 5.6 years; IQR 2.9–7.0) were available. Of 17 positive draws, 6 were positive at the next draw (35.3%; 17.3–58.7); of 523 negative draws, 25 became positive (4.8%; 3.3–7.0), or 0.89 new positives per 100 person-years. In LSADT, the single 1997 positive was negative in 2007, and 7 of 85 negatives became positive. Technical replicates agreed in 8/8 LSADT pairs and 36/36 GSE55763 pairs; all were negative, so agreement among positives is untested.

3.3 CHIP is not expected to produce a positive call on its own

All 48 loci were present in the CHIP meta-analysis[6]. Ten loci were associated with any CHIP at $P < 1.04\times10^{-3}$ (5 toward and 5 away from the panel direction); for DNMT3A CHIP, 9 (2 toward, 7 away); for TET2 CHIP, 1 (toward). The largest absolute carrier–non-carrier difference was 0.048 β (TET2 CHIP at CUX1 cg18088486, a non-evaluable locus; away from the panel direction; P = 0.27). The largest difference toward the panel direction was 0.032 β (TET2 CHIP at RUNX1 cg00994804; P = 0.06, not significant); scaled to a 60% clone, it corresponds to 0.096 β, below the 0.20 β criterion at every locus. DNMT3A CHIP lowers methylation at six WT1 loci whose disease direction is gain, moving carriers away from a positive call (Fig. 2).

3.4 What a positive result should trigger

Table 3 and Figs. 3–4 summarize the decision model for 70–79 years at an assumed sensitivity of 0.50. In unselected adults (prevalence 0.09% at a 2-year undiagnosed phase), 25 per 1,000 screened would be positive and 0.4 would have disease (PPV 1.8%; 0.9–4.1); net benefit was −0.06 per 1,000 at a 2% threshold and negative above it. A repeat draw reduced positives to 9 per 1,000 (PPV 4.8%) and net benefit to +0.26 per 1,000 at 2% and −0.02 at 5%. In CHIP carriers, PPV was 9.2% (4.7–18.9) at a prevalence of 0.5% and 29% (17–50) at 2.0%; with a repeat draw it rose to 54% (32–78), reducing work-ups per 1,000 from 34 to 19 at the same number of detections. With a repeat draw, net benefit in CHIP carriers was positive at every threshold from 1% to 10% in every prevalence and sensitivity scenario; one-step testing turned negative at a 10% threshold when prevalence was 0.5–1% and sensitivity 0.25–0.50. Under pessimistic repeat assumptions (repeat-positive probability 0.75 among false positives; repeat sensitivity equal to first-draw sensitivity), the lowest scenario (prevalence 0.5%, sensitivity 0.25) was negative from a 2% threshold. At 80 years or older, at a CHIP prevalence of 0.5% and a 5% threshold, net benefit was −1.4 per 1,000 for one-step and +1.1 per 1,000 for two-step testing.

3.5 Pre-diagnostic data

The released GSE253126 data contains 5,290 candidate regions (0.17% of the genome) selected by the authors as differing between future cases and controls. No panel CpG lay within 2 kb of any region; three lay within 10 kb, all at non-evaluable loci. With 0.08 overlaps expected by chance, the result is uninformative about pre-diagnostic signal.

4 Discussion

Three findings define where this readout could be used. First, its false-positive rate is low below 80 years—about 2–3 per 100 in the seventh and eighth decades—but triples after 80. Second, a third of positives persist over years, so a repeat draw removes most but not all of them. Third, published CHIP effects at the panel loci are two- to fourfold too small to cross the call threshold on their own, so CHIP status is not expected to inflate the false-positive rate substantially; the age gradient more plausibly reflects age-related drift, composition change, or clonal events other than DNMT3A/TET2 CHIP, which these data cannot separate.

Together, these results argue against the pathway the original framing implied. In unselected older adults, the prevalence of preclinical myeloid neoplasm is so low that, at the observed false-positive rates, PPV stays below 5% even with a repeat draw at an assumed sensitivity of 0.50 and an undiagnosed phase of 1–2 years (it exceeds 5% under the 5-year scenario or at sensitivity 0.75); a reflex mutation panel triggered by this readout would mostly confirm CHIP, which is common and usually benign[2,3]. In known CHIP carriers—already sequenced, with a measured progression risk of 0.5–1% per year—the same false-positive rates give PPVs of 9–54% at an assumed

sensitivity of 0.50, depending on prevalence and strategy, and positive net benefit. The appropriate action in that group is hematologic work-up, not another mutation test. We therefore propose testing the readout as a stratification step within CHIP surveillance, with a confirmatory repeat draw before work-up and mandatory at 80 years or older. A decision-support layer for this pathway (LENA-Rx) is in development; no performance is claimed for it here.

These conclusions depend on sensitivity, which remains unmeasured. The only public pre-diagnostic blood methylation dataset we found does not cover the panel loci in its released form[4], and disease cohorts measured at diagnosis would overstate early sensitivity. The decision model therefore shows where the readout could be useful under stated assumptions, not that it is. The required study is prospective or nested within a CHIP cohort with stored blood and adjudicated outcomes.

4.1 Limitations

- Sensitivity before diagnosis is assumed (0.25–0.75), not measured.
- No cohort had individual CHIP labels; the CHIP contribution is inferred from carrier-average summary statistics, assumes linear scaling with clone size, and excludes ASXL1 magnitudes (not on the β scale in the deposit).
- The ≥80 estimate rests on 160 individuals from two Nordic twin cohorts; LSADT ages were assigned by wave, so some 1997 samples (age 80–82) are mis-banded.
- Leukocyte composition was not adjusted for the primary call; age-related shifts in composition remain a competing explanation for the age gradient.
- The CHIP progression input (≈0.5%/y overall, ≈1%/y at VAF > 0.10) comes from 16 incident hematologic cancers, 5 in carriers, across two cohorts[2], and counts all hematologic cancers, lymphoid included; it is imprecise and may overstate myeloid-specific progression, which would inflate the CHIP-carrier PPVs.
- Persistence was measured over years; persistence over a weeks-long confirmatory interval is likely higher and would reduce the benefit of a repeat draw.
- Prevalence inputs come from US registries, which undercount MDS[18], and so may understate prevalence in unselected adults; the 5-year scenario partly covers this.
- All cohorts used the 450K array; transfer to a targeted assay requires separate analytical validation.
- Nine analysis changes were made after results were seen (Supplementary Table S2).

5 Conclusions

A fixed 48-locus whole-blood methylation burden call has false-positive rates of about 2% in the seventh and eighth decades and 7.5% after 80, one-third of which persist. Its plausible clinical use is in known CHIP carriers, with a confirmatory repeat draw before hematologic work-up, not as a trigger for reflex mutation testing in unselected older adults. Pre-diagnostic sensitivity must be measured before any clinical use.

Declarations

Funding. Alercell, Inc. No external funding.

Conflicts of interest. F.S. is an officer and majority shareholder of Alercell, Inc., which develops DNA-methylation diagnostics for hematologic malignancy and has filed a U.S. provisional patent application (64/113,991) covering the panel.

Ethics approval and consent. Not applicable: analysis of public, de-identified data under the original studies' approvals and data-use terms.

Data availability. GSE87571, GSE42861, GSE73115, GSE55763 and GSE253126 (NCBI GEO); E-MTAB-7309 (ArrayExpress); Kirmani et al. summary statistics (Zenodo 10.5281/zenodo.14712757). Per-sample scores for all cohorts, the reference file (per-locus median and SD from GSE87571) and a standalone scoring script that reproduces every count reported here are deposited on Zenodo (doi:10.5281/zenodo.24094222).

Code availability. The standalone scoring script is deposited with the data (doi:10.5281/zenodo.24094222).

Author contributions. F.S. conceived the study, performed the analyses, and wrote the manuscript.

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Tables

Table 1. Cohorts. All test cohorts except GSE55763 were processed from raw IDATs through the same SeSAMe pipeline as the reference.

Cohort	Accession	n (arrays / individuals)	Age, years	Role
GSE87571	GEO	732 / 732	14–94	Reference only (median, SD per locus)
Rheumatoid arthritis controls	GSE42861	335 / 335	20–70 (median 55)	A1
SATSA	E-MTAB-7309	1,071 / 444	48–98 at baseline (median 71.6)	A1 (baseline), A2 (540 intervals)
LSADT	GSE73115	180 / 86 (+8 replicate arrays)	73–82 in 1997; 10 y later in 2007	A1, A2, technical replicates
Lehne et al.	GSE55763	2,711 / 2,639 population + 36 replicate pairs	24–75	Sensitivity analysis (processed data)
Kirmani et al.	Zenodo 14712757	N = 8,196 (summary statistics)	—	A3
Lao et al.	GSE253126	233 (66 future AML/MDS, 167 controls)	—	A6 (exploratory)

Table 2. Positive calls (≥4 of 40 loci shifted ≥0.20 β) by age band and cohort, baseline samples. Pooled column: Wilson 95% CI.

Age band	GSE42861	SATSA	LSADT	Pooled
<60	0/230	1/78	—	1/308 = 0.3% (0.1–1.8)
60–69	2/100	2/108	—	4/208 = 1.9% (0.8–4.8)
70–79	1/4	4/151	1/86 (1997 wave)	6/241 = 2.5% (1.1–5.3)
≥80	—	5/74	7/86 (2007 wave)	12/160 = 7.5% (4.3–12.7)
Baseline total	3/334 = 0.9% (0.3–2.6)	12/411 = 2.9% (1.7–5.0)	1/86 = 1.2% (0.2–6.3)	16/831 = 1.9% (1.2–3.1)

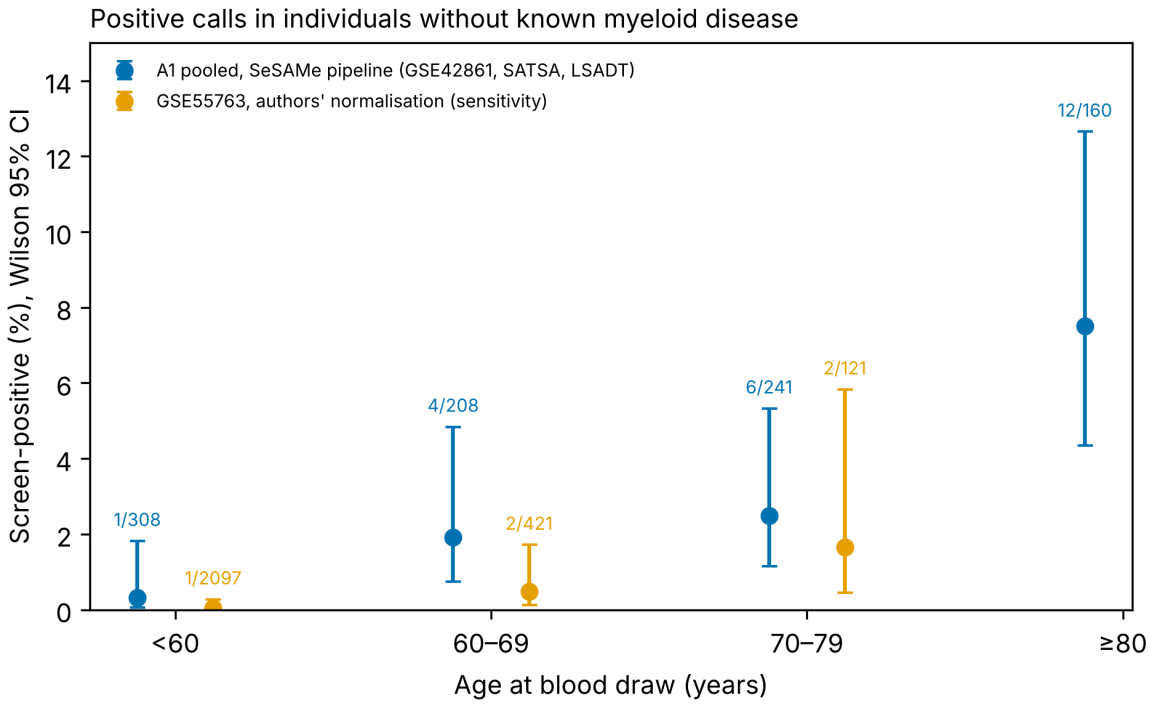
The ≥80 row combines SATSA baseline and the LSADT 2007 wave; the baseline total uses LSADT 1997 only.

Table 3. Decision model, 70–79 years, assumed sensitivity 0.50, per 1,000 screened. UI, 95% uncertainty interval from 10,000 draws.

Population (prevalence)	Strategy	Positives	True detections	PPV (95% UI)	Net benefit, 2% threshold
Unselected ≥60 (0.09%)	One-step	25	0.4	1.8% (0.9–4.1)	≤ 0
Unselected ≥60 (0.09%)	Two-step	9	0.4	4.8% (2.0–14.0)	≈ 0
CHIP carriers (0.5%)	One-step	27	2.5	9.2% (4.7–18.9)	> 0
CHIP carriers (2.0%)	One-step	34	10	29% (17–50)	> 0
CHIP carriers (2.0%)	Two-step	19	10	54% (32–78)	> 0

Two-step: repeat draw; work-up only if both positive; repeat-positive probability among false positives 6/17, disease assumed to remain positive. Full grid (all age bands, sensitivities, prevalence scenarios and repeat assumptions) in Supplementary Data.

Figures



Positive call: ≥4 of 40 evaluable loci shifted ≥0.20 β toward the panel direction vs GSE87571 reference (n = 732).

Fig. 1. Proportion of samples called positive by age band, with Wilson 95% CIs and counts. Blue: baseline samples from GSE42861, SATSA and LSADT processed through one SeSAMe pipeline. Orange: GSE55763, authors' normalization, shown as a sensitivity analysis (no participants aged ≥80).

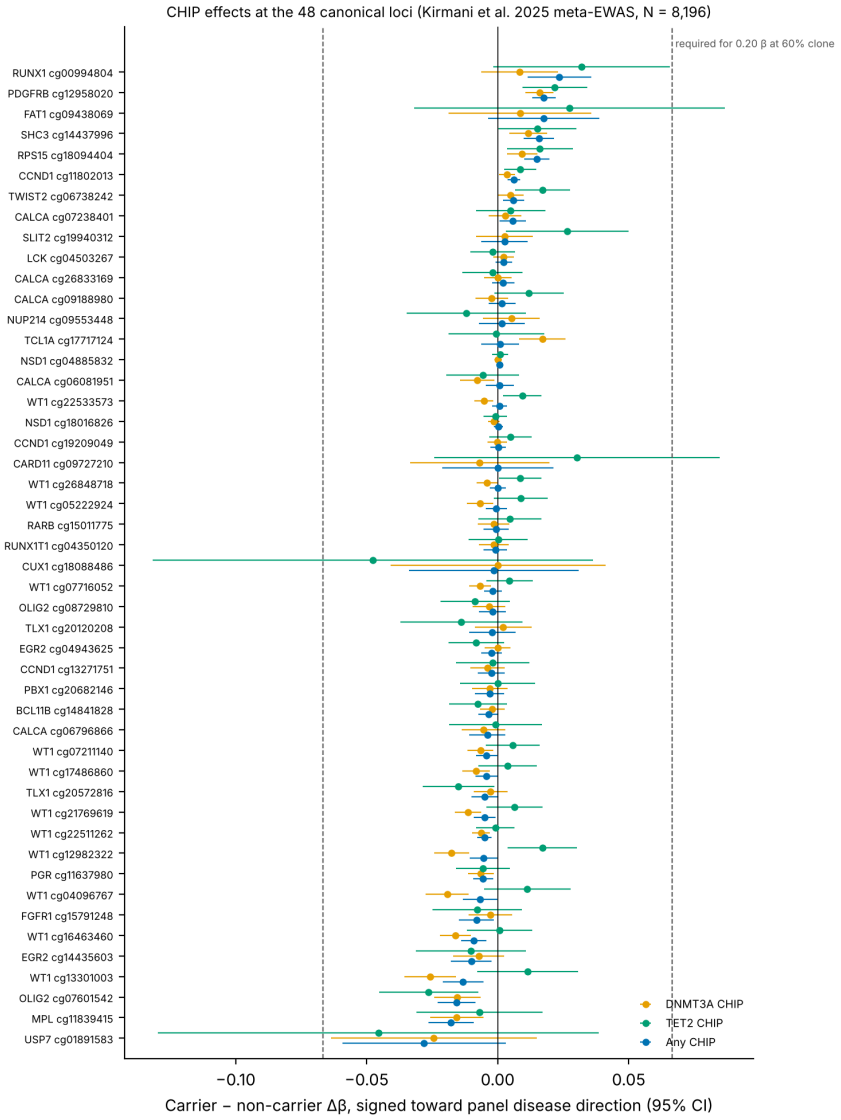


Fig. 2. Carrier minus non-carrier β difference at the 48 panel loci for any, DNMT3A and TET2 CHIP (Kirmani et al.[6]; N = 8,196), signed so that positive values point toward the panel's disease direction; bars, 95% CI. Dashed lines: average carrier shift that would reach 0.20 β at a 60% clone if the carrier-average clone fraction is 0.2.

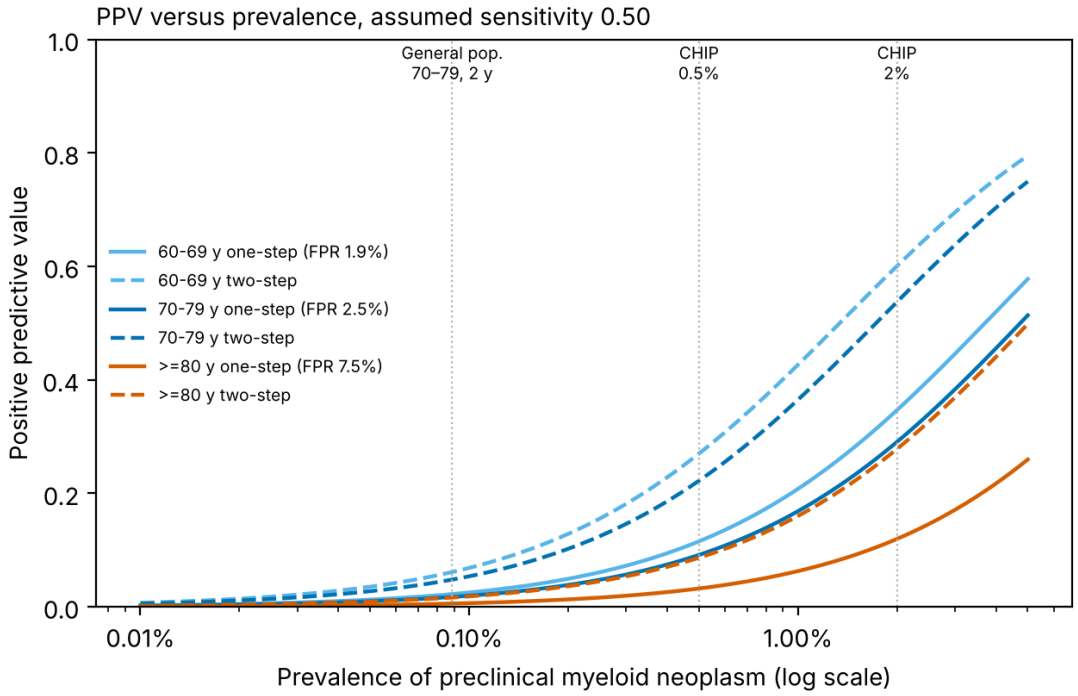
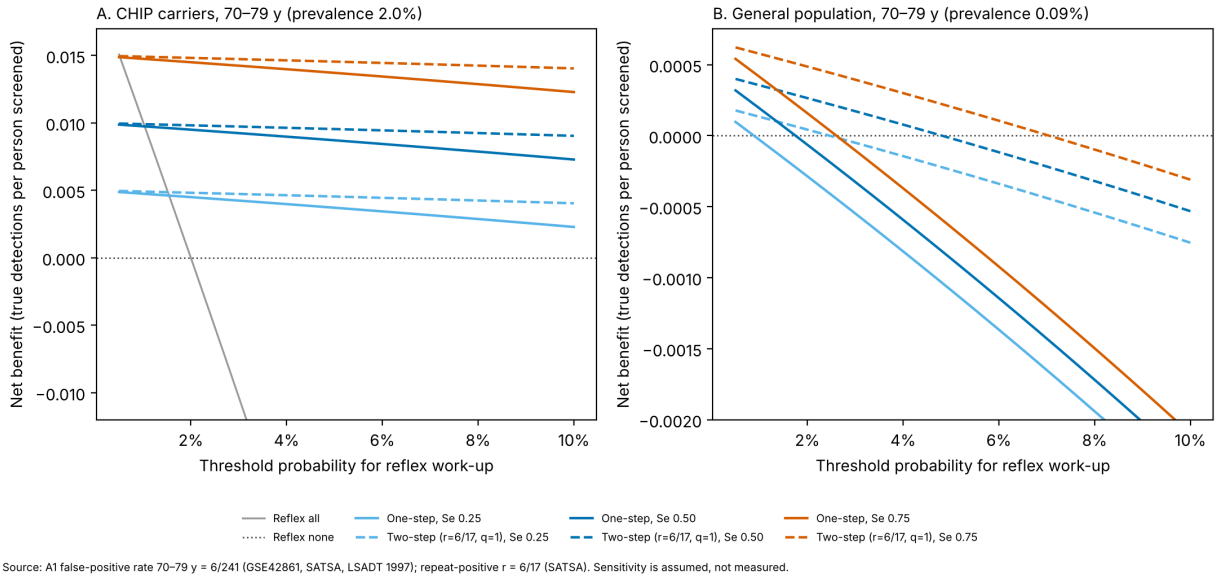


Fig. 3. Positive predictive value against prevalence of preclinical myeloid neoplasm, by age band, for one-step (solid) and two-step (dashed) strategies at an assumed sensitivity of 0.50. Vertical lines mark the unselected 70–79 scenario (0.09%) and CHIP-carrier scenarios (0.5% and 2%).



Source: A1 false-positive rate 70–79 y = 6/241 (GSE42861, SATSA, LSADT 1997); repeat-positive r = 6/17 (SATSA). Sensitivity is assumed, not measured.

Fig. 4. Decision curves, 70–79 years. (A) CHIP carriers, prevalence 2.0%. (B) Unselected adults, prevalence 0.09%. Solid: one-step; dashed: two-step (repeat-positive probability 6/17). Grey: work up everyone; dotted: work up no one. Sensitivity is assumed.

Supplementary Table S1

The 48 panel loci. Position: GRCh38, 1-based CpG position (SeSAMe HM450 manifest, GENCODE v22). Context: CpG island, shore, shelf or open sea. Direction: expected change in disease. Reference median and SD: GSE87571, n = 732. NE, non-evaluable (masked in >10% of reference arrays).

Gene	Probe	GRCh38	Context	Direction	Ref. median β	Ref. SD
BCL11B	cg14841828	chr14:99262751	OpenSea	hyper	0.045	0.063
CALCA	cg06081951	chr11:14969867	N_shelf	hyper	0.446	0.047
CALCA	cg06796866	chr11:14973098	N_shore	hyper	0.380	0.052
CALCA	cg07238401	chr11:14972851	N_shore	hyper	0.527	0.042
CALCA	cg09188980	chr11:14971832	N_shore	hyper	0.327	0.048
CALCA	cg26833169	chr11:14972499	S_shore	hyper	0.277	0.035
CARD11	cg09727210	chr7:2922606	OpenSea	hypo	NE	—
CCND1	cg11802013	chr11:69654407	S_shore	hypo	0.941	0.020
CCND1	cg13271751	chr11:69640034	island	hyper	NE	—
CCND1	cg19209049	chr11:69640061	island	hyper	0.090	0.028
CUX1	cg18088486	chr7:102193868	N_shore	hyper	NE	—
EGR2	cg04943625	chr10:62818371	island	hyper	0.170	0.031
EGR2	cg14435603	chr10:62818426	island	hyper	0.261	0.077
FAT1	cg09438069	chr4:186659939	OpenSea	hyper	NE	—
FGFR1	cg15791248	chr8:38469212	S_shore	hyper	0.743	0.056
LCK	cg04503267	chr1:32276436	S_shore	hypo	0.899	0.033
MPL	cg11839415	chr1:43349093	island	hyper	0.816	0.078
NSD1	cg04885832	chr5:177133264	island	hyper	0.057	0.011
NSD1	cg18016826	chr5:177133000	island	hyper	0.064	0.015
NUP214	cg09553448	chr9:131125037	N_shore	hyper	0.184	0.074
OLIG2	cg07601542	chr21:33024678	island	hyper	0.357	0.055
OLIG2	cg08729810	chr21:33024636	island	hyper	0.192	0.043
PBX1	cg20682146	chr1:164576606	island	hyper	0.615	0.044
PDGFRB	cg12958020	chr5:150129831	N_shelf	hypo	0.921	0.060
PGR	cg11637980	chr11:101129986	S_shore	hyper	NE	—
RARB	cg15011775	chr3:25441446	OpenSea	hypo	NE	—
RPS15	cg18094404	chr19:1438018	N_shore	hypo	NE	—
RUNX1	cg00994804	chr21:34887086	island	hypo	0.456	0.112
RUNX1T1	cg04350120	chr8:91992056	OpenSea	hypo	0.801	0.055
SHC3	cg14437996	chr9:89179772	S_shore	hypo	0.551	0.058
SLIT2	cg19940312	chr4:20255967	S_shore	hyper	0.275	0.079
TCL1A	cg17717124	chr14:95714510	island	hyper	0.265	0.060
TLX1	cg20120208	chr10:101137112	S_shore	hypo	0.323	0.085
TLX1	cg20572816	chr10:101136345	N_shore	hyper	0.197	0.039
TWIST2	cg06738242	chr2:238847062	island	hyper	0.206	0.048
USP7	cg01891583	chr16:8902069	OpenSea	hyper	NE	—
WT1	cg04096767	chr11:32427904	island	hyper	0.384	0.059
WT1	cg05222924	chr11:32428940	S_shore	hyper	0.151	0.037
WT1	cg07211140	chr11:32433479	island	hyper	0.129	0.043
WT1	cg07716052	chr11:32433202	N_shore	hyper	0.261	0.036
WT1	cg12982322	chr11:32428275	S_shore	hyper	0.455	0.047

Gene	Probe	GRCh38	Context	Direction	Ref. median β	Ref. SD
WT1	cg13301003	chr11:32427708	N_shore	hyper	0.539	0.070
WT1	cg16463460	chr11:32433172	N_shore	hyper	0.381	0.039
WT1	cg17486860	chr11:32433294	N_shore	hyper	0.133	0.037
WT1	cg21769619	chr11:32433222	N_shore	hyper	0.252	0.031
WT1	cg22511262	chr11:32433646	island	hyper	0.126	0.025
WT1	cg22533573	chr11:32431225	S_shore	hyper	0.166	0.028
WT1	cg26848718	chr11:32433429	island	hyper	0.072	0.035

Supplementary Table S2

Changes made after the analysis plan was locked (all post hoc).

#	Change
1	Intended use framed as two populations (CHIP carriers → hematologic work-up; unselected adults → reflex panel) instead of unselected adults only.
2	35-locus sensitivity analysis removing five loci with excess masking in SATSA.
3	LSADT replicate “-1” used; ages assigned by wave.
4	SATSA baseline estimate reported as primary and the all-array estimate as secondary (as locked; an earlier working value used all arrays).
5	Crude pooling plus cohort-adjusted GEE instead of random-effects pooling (sparse cells).
6	Composition adjustment for the secondary rule only.
7	A6 analysed as locus–region overlap because released regions do not cover the panel.
8	GSE55763 masked with the authors' detection P values.
9	Progression input sourced to Jaiswal 2014; MDS incidence interpolated between published bands.

Supplementary Files

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- STROBEChecklistMDTv7.docx