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1 **Letter to the editor (1500 words)**

2

3 **Clinical relevance of failed and missing cytogenetic analysis in acute myeloid leukaemia (AML)**

4

5 Chromosomal and genetic abnormalities are important prognostic factors in AML and most clinically
6 relevant aberrations are detectable by cytogenetic analysis.(1, 2) One limitation of cytogenetics is
7 failure due to a lack of analysable metaphases. Despite this shortcoming, chromosomal analysis
8 remains the gold standard test for identifying abnormalities used to risk-stratify treatment because
9 many abnormalities (e.g. those involving large chromosomal regions and a complex karyotype) can
10 only be described in cytogenetic terms. Thus, failure to obtain a cytogenetic result impacts on risk
11 stratification. In support of this suggestion, two recent reports concluded that failed and/or missing
12 cytogenetic results were associated with an adverse prognosis.(3, 4) Our view is that assignment of
13 risk on the basis of the absence of information is counterintuitive and potentially problematic.
14 Therefore, we investigated the distribution and prognostic impact of failed and missing cytogenetic
15 results in successive MRC AML trials.

16

17 Cytogenetic analysis of pre-treatment bone marrow or peripheral blood samples was performed
18 locally, reviewed and collated by the Leukaemia Research Cytogenetics Group. Results were
19 available from 10,685 patients (1-82 years old) recruited to successive trials (AML12, AML14, AML15,
20 AML16) between 1995-2012.(1, 5-9) At diagnosis, patients recruited to AML14 and AML16 were
21 classified, on the basis of presenting features, as suitable or unsuitable for intensive therapy. All
22 studies were approved by the relevant ethics committees and informed consent was obtained in
23 accordance with the Declaration of Helsinki.

24

25 Karyotypes were described according to ISCN.(10) If the regional cytogenetic laboratory received a
26 sample within the diagnostic window (30 days prior to or 7 days after diagnosis) cytogenetic testing
27 was deemed to have been attempted (“Sample”); otherwise cytogenetic analysis was classified as
28 missing (“No sample”). Analysis was defined as “Successful” if a clonal chromosomal abnormality
29 was detected or ≥ 20 normal metaphases were fully analysed; otherwise it was classified as
30 “Failed”.(11)

31

32 Survival was calculated from trial entry to death or last follow-up. Patients were censored at
33 31/10/2010 (AML12, AML14) or 01/01/2012 (AML15, AML16) when follow-up was complete for 95%
34 of the patients. Survival rates were calculated and compared using the Kaplan-Meier method, log-
35 rank test, and Cox regression model. Comparisons between groups were performed using logistic
36 regression, χ^2 test or Wilcoxon rank-sum test. Multivariate logistic regression was used to determine
37 predictors for missing and failed cytogenetics. As recruitment and eligibility changed by trial and
38 diagnosis period, all odds (OR) and hazard (HR) ratios were adjusted for intensive (INT) versus non-
39 intensive (NI) treatment and year of diagnosis. All P-values were two-tailed. Statistical analyses were
40 performed using SAS v9.3 (SAS Institute Inc., Cary, NC, USA).

41

42 Cytogenetic analysis was attempted in 94% INT patients but in only 83% NI patients ($p < 0.0001$).
43 Among INT patients, cytogenetic analysis was attempted more frequently among patients for whom
44 the result would affect treatment; that is, younger patients treated on AML12 or AML15 (Table 1).
45 This correlated with the observation that cytogenetic uptake was lower, and did not vary by age or
46 trial, among NI patients. Similarly, cytogenetic uptake was higher among patients with de novo or
47 therapy-related AML than for those with an antecedent hematologic disease. These patients were
48 likely to have had cytogenetic analysis carried out at the time of initial diagnosis and subsequent
49 testing may have been deemed unnecessary. Surprisingly, the uptake of cytogenetic analysis

50 decreased marginally across successive trials and by period of diagnosis. However, it should be
51 noted that recruitment rates and patterns changed significantly over this period with a greater
52 number of smaller regional hospitals participating in later trials. Across the whole cohort,
53 multivariate analysis revealed that secondary disease (OR 2.08 (95% confidence interval 1.73-2.49),
54 $p < 0.0001$), white blood cell count (WBC) (0.82 per 10-fold increase (0.74-0.92), $p = 0.0002$), and age
55 (1.10 per decade (1.04-1.16) $p = 0.0009$) were the most significant predictors of cytogenetic testing.
56 Similar results were obtained when INT and NI patients were examined separately; although age was
57 not significant in the latter group (Table 1). Among INT patients, a lack of cytogenetic testing was
58 associated with an inferior OS: 27% v 38%, HR = 1.41 (1.26-1.58), $p < 0.0001$ (Figure 1A). However,
59 this effect was restricted to younger adults (OS 35% v 45%, 1.41 (1.20-1.64), $p < 0.0001$), and not
60 observed among children (65% v 66%, 0.95 (0.43-2.09), $p = 0.9$) or older adults (11% v 14%, 1.07
61 (0.92-1.24), $p = 0.4$) (Figure 1B) (p value for heterogeneity = 0.01). Similar results were obtained when
62 the analysis was adjusted for age, WBC, secondary disease and performance status.

63

64 The frequency of cytogenetic testing among NI patients was similar to the Swedish study (4) which
65 excluded NI patients (83% v 80%). Interestingly, cytogenetics was not used to guide therapy in
66 Sweden during the study timeframe, which may explain the low uptake of cytogenetic testing;
67 similar to the rate among NI patients in this study. Lazarevic et al concluded that patients without
68 cytogenetic testing had an inferior outcome; similar to that for high risk cytogenetic patients.
69 However, the survival of patients with and without cytogenetic testing was similar (28% v 22%). In
70 contrast, we found that the association between inferior outcome and the uptake of cytogenetics
71 was only significant among younger adults. Moreover, among younger adults the survival of patients
72 without cytogenetic testing (35%) was closer to those with intermediate rather than high risk
73 cytogenetics (33% and 12%, respectively).(1) In this study, a lack of cytogenetic testing was
74 associated with other high risk features (age and secondary disease) which are established
75 prognostic factors. Although cytogenetic testing was associated with an inferior outcome in

76 multivariable analyses (HR 1.13 (1.01-1.23) $p=0.04$) the size of the effect was diminished indicating
77 that other factors like secondary disease are also important. The Swedish study did not report the
78 frequency of secondary disease and it is likely to be higher in a population-based study than a clinical
79 trial. Collectively these findings indicate that there are numerous factors governing the uptake of
80 cytogenetic testing at the time of diagnosis; many of which are also likely to impact on survival. Also,
81 there are likely to be additional factors that cannot be examined in centralised retrospective studies.

82

83 A successful cytogenetic result was obtained in 90% cases and there was no difference according to
84 treatment intensity (Table 1). Among NI patients, there were no significant predictors of cytogenetic
85 failure whereas age and increasing WBC correlated with higher cytogenetic failure rates among INT
86 patients. The variation in failure rate by trial was linked to age as AML14 and AML16, trials for older
87 adults, had the highest failure rates. Multivariate logistic regression analysis revealed that the key
88 predictor of cytogenetic failure was age (OR 1.14 per decade (1.09-1.19), $p<0.0001$) and, to a lesser
89 extent, WBC (OR 1.11 per 10-fold increase (1.01-1.22), $p=0.04$). The link between age and
90 cytogenetic failure could be explained by the increasing frequency of normal karyotype with age(12)
91 and the fact that the threshold used to distinguish normal and failed cytogenetic result has shifted
92 over time.(11) The link between cytogenetic failure and high WBC may be due to overcrowding of
93 accumulated blasts within the bone marrow leading to inhibition of cell division, an observation
94 which has often been made within routine preparation of leukaemic samples (unpublished
95 observation). There was no association between cytogenetic failure and survival either overall
96 (Figure 1C) or within different age groups for INT or NI patients. The OS rates for children, young
97 adults and older adults treated intensively with successful and failed cytogenetics was: 38% v 37%,
98 1.04 (0.95-1.13), $p=0.4$; 45% v 48%,
99 0.94 (0.84-1.05), $p=0.3$; 15% v 13%, 0.99 (0.87-1.12), $p=0.9$, respectively. In contrast, the SWOG and
100 Swedish studies (3, 4) concluded that cytogenetic failure was associated with an inferior outcome.

101 However, they compared patients whose samples failed cytogenetic testing to those stratified by
102 cytogenetic risk. There is no biological reason why patients with failed cytogenetics should differ
103 from those with successful cytogenetics; in fact there is evidence to the contrary.(13) Hence a
104 successful versus failed comparison is the most informative analysis. The survival of patients with
105 successful and failed cytogenetics in the SWOG and Swedish studies were not very different (21% v
106 16% and 28% v 25% respectively). Cytogenetic failure was higher in our study (~10%) than the SWOG
107 and Swedish studies (6% and 3%) because we used a definition based on the likelihood of detecting
108 a clonal chromosomal abnormality.(11) Applying this stricter definition would move cases from the
109 intermediate risk to the failed category; hence would not have altered the conclusions from the
110 other studies. The factors governing cytogenetic failure are not fully understood but sample
111 transport and processing are likely to be more important than underlying biological factors.(13, 14)
112 Hence there is no rationale as to why cytogenetic failure should be linked to outcome.

113

114 Given the importance of genetics in guiding therapy in AML, the reasons for not sending a sample for
115 analysis do warrant further investigation; but this must be done prospectively and more detailed
116 information about the diagnostic environment needs to be collected. The results of this large study
117 coupled with a re-examination of the previous studies do not support the conclusion that missing
118 nor failed cytogenetics are reliable or, indeed, appropriate prognostic markers.

119

120

121 **Conflict-of-interest disclosure**

122 The authors declare no competing financial interests.

123

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130

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132 A.V.M analysed and interpreted data. D.G. and R.K.H. provided clinical and follow-up data; A.K.B.
133 was the Chief Investigator on all trials. C.J.H. and A.V.M. provided financial and administrative
134 support. A.V.M wrote the manuscript with input and approval from all other authors.

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212 Table and Figure Legends

213 Table 1: Demographics and clinical features for 10,685 patients treated on consecutive UK MRC
214 acute myeloid leukaemia trials.

215 Figure 1: Overall survival of MRC AML intensively treated patients according the presence or absence
216 of cytogenetic analysis (A) and for older adults (B) and by the success of cytogenetic analysis (C).

217 Survival rates are at 5 years for intensively treated patients.

218

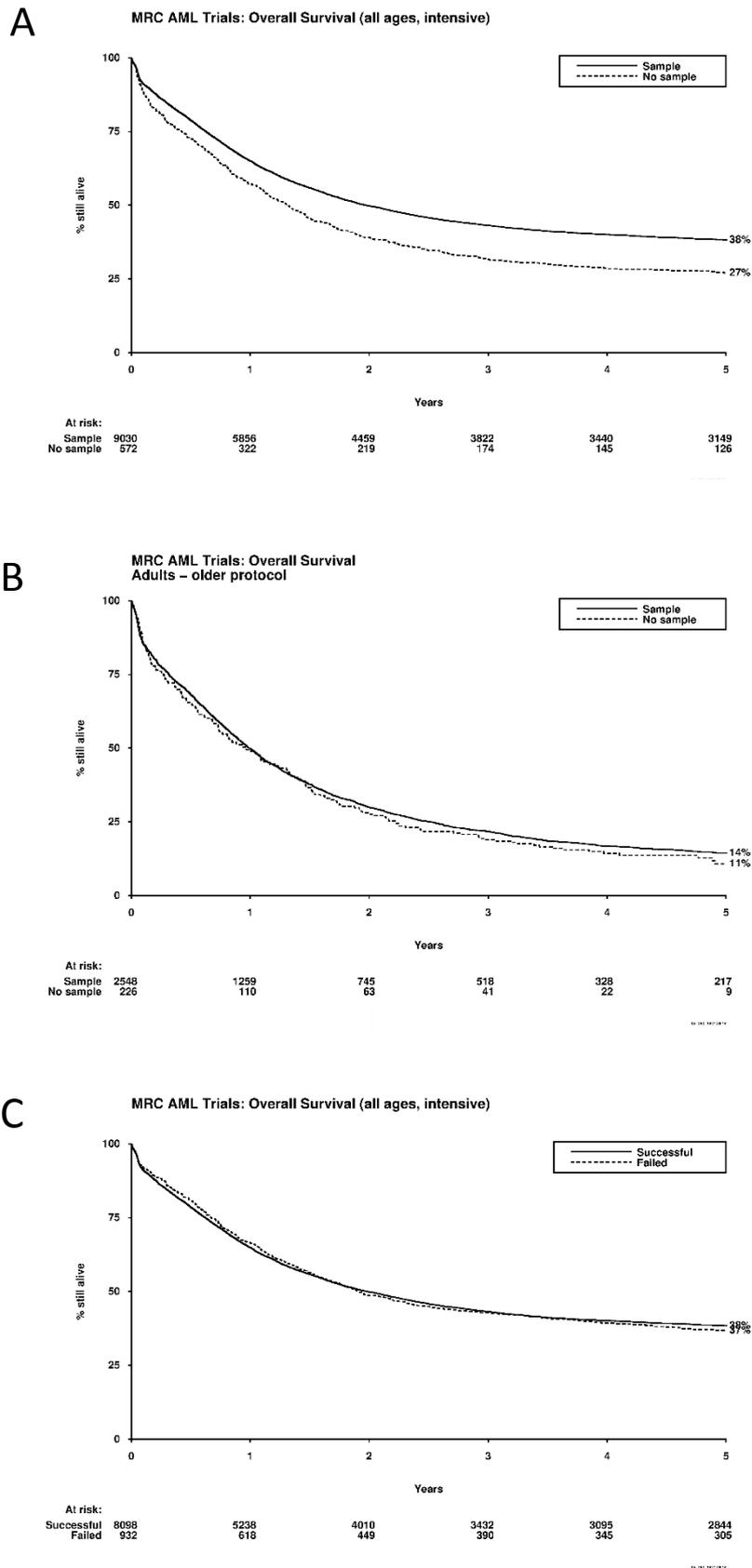


Figure 1: Overall survival of MRC AML intensively treated patients according the presence or absence of cytogenetic analysis (A) and for older adults (B) and by the success of cytogenetic analysis (C). Survival rates are at 5 years for intensively treated patients.

Table 1: Demographics and clinical features for 10,685 patients treated on consecutive UK MRC acute myeloid leukaemia †

		Intensively treated patients						Non-intensively treated patients					
		No sample	Sample	<i>p</i>	Successful	Failed	<i>p</i>	No sample	Sample	<i>p</i>	Successful	Failed	<i>p</i>
Total, n (%)		583 (6)	9085 (94)		8151 (90)	934 (10)		176 (17)	841 (83)		754 (90)	87 (10)	
Sex, n (%)	Female	260 (6)	4175 (94)	0.5	3754 (90)	421 (10)	0.6	61 (15)	347 (85)	0.1	304 (88)	43 (12)	0.1
	Male	323 (6)	4910 (94)		4397 (90)	513 (10)		115 (19)	494 (81)		450 (91)	44 (9)	
Age (years), n (%) [*]	<15	21 (3)	722 (97)	<.0001	677 (94)	45 (6)	<.0001	-	-	0.3	-	-	0.8
	15-29	46 (5)	899 (95)		832 (93)	67 (7)		-	-		-	-	
	30-39	39 (4)	1016 (96)		919 (90)	97 (10)		1 [^]	0		1 [^]	0	
	40-49	64 (4)	1416 (96)		1266 (89)	150 (11)		-	-		-	-	
	50-59	133 (6)	2061 (94)		1857 (90)	204 (10)		4 (40)	6 (60)		5 (83)	1 (17)	
	60-69	190 (8)	2190 (92)		1929 (88)	261 (12)		37 (22)	129 (78)		115 (89)	14 (11)	
	70-79	88 (10)	768 (90)		661 (86)	107 (14)		97 (15)	546 (85)		490 (90)	56 (10)	
	80+	2 (13)	13 (87)		10 (77)	3 (23)		38 (19)	159 (81)		143 (90)	16 (10)	
WBC x10 ⁹ /L [*] , n (%)	0-9.9	329 (7)	4600 (93)	0.0003	4146 (90)	453 (10)	0.0001	110 (19)	465 (81)	0.12	412 (89)	53 (11)	0.6
	10-49.9	166 (6)	2505 (94)		2276 (91)	229 (9)		45 (15)	252 (85)		231 (92)	21 (8)	
	50-99.9	38 (4)	971 (96)		858 (88)	113 (12)		15 (15)	86 (85)		78 (91)	8 (9)	
	100+	43 (5)	911 (95)		779 (86)	132 (14)		6 (14)	37 (86)		33 (86)	5 (14)	
Performance Status [*] , n (%)	0	366 (6)	5593 (94)	0.9	5014 (90)	579 (10)	0.2	65 (19)	275 (81)	0.3	246 (89)	30 (11)	>0.95
	1	168 (6)	2603 (94)		2356 (91)	247 (9)		87 (17)	424 (83)		383 (90)	41 (10)	
	2+	46 (6)	770 (94)		668 (87)	102 (13)		24 (15)	141 (85)		125 (89)	16 (11)	
Diagnosis, n (%)	De Novo	455 (5)	8170 (95)	<.0001	7340 (90)	830 (10)	0.3	111 (15)	627 (85)	0.002	556 (89)	71 (11)	0.11
	Secondary	128 (12)	915 (88)		811 (89)	104 (11)		65 (23)	214 (77)		198 (93)	16 (7)	
Type of secondary, n (%)	AHD	95 (14)	607 (86)	0.04; 0.01 ^{**}	542 (89)	65 (11)	0.03; 0.16 ^{**}	13 (20)	53 (80)	0.5; 0.4 ^{**}	141 (94)	9 (5)	0.3;0.12 ^{**}
	t-AML	5 (5)	98 (95)		92 (94)	6 (6)		50 (25)	150 (75)		9 (82)	2 (18)	
	Not stated	28 (12)	210 (88)		177 (84)	33 (16)		2 (15)	11 (85)		48 (91)	5 (9)	
Trial, n (%)	AML12	134 (4)	3270 (96)	<.0001	2982 (91)	288 (9)	<.0001	-	-	0.03	-	-	0.5
	AML14	78 (7)	1044 (93)		887 (85)	157 (15)		36 (13)	239 (87)		217 (91)	22 (9)	
	AML15	219 (6)	3259 (94)		2941 (90)	318 (10)		-	-		-	-	
	AML16	152 (9)	1512 (91)		1341 (89)	171 (11)		140 (19)	602 (81)		537 (89)	65 (11)	
Period of diagnosis [*] , n (%)	1995-99	89 (4)	2193 (96)	<.0001	1996 (91)	197 (9)	0.15	1 (3)	37 (97)	0.005	29 (78)	8 (22)	0.7
	2000-04	189 (6)	3100 (94)		2760 (89)	340 (11)		25 (15)	146 (85)		137 (94)	9 (6)	
	2005-09	238 (7)	3100 (93)		2776 (90)	324 (10)		99 (17)	468 (83)		417 (89)	51 (11)	
	2010-12	67 (9)	692 (91)		619 (89)	73 (11)		51 (21)	190 (79)		171 (90)	19 (10)	

^{*} test for trend; ^{**} excluding not stated. Some children did not have WHO PS (not valid); [^] This 36 year old patient was deemed unsuitable for non-intensive treatment and was treated on AML16