Letter to the editor (1500 words)

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

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

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

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

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

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

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

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

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

A successful cytogenetic result was obtained in 90% cases and there was no difference according to treatment intensity (Table 1). Among NI patients, there were no significant predictors of cytogenetic failure whereas age and increasing WBC correlated with higher cytogenetic failure rates among INT patients. The variation in failure rate by trial was linked to age as AML14 and AML16, trials for older adults, had the highest failure rates. Multivariate logistic regression analysis revealed that the key predictor of cytogenetic failure was age (OR 1.14 per decade (1.09-1.19), p<0.0001) and, to a lesser extent, WBC (OR 1.11 per 10-fold increase (1.01-1.22), p=0.04). The link between age and cytogenetic failure could be explained by the increasing frequency of normal karyotype with age(12) and the fact that the threshold used to distinguish normal and failed cytogenetic result has shifted over time.(11) The link between cytogenetic failure and high WBC may be due to overcrowding of accumulated blasts within the bone marrow leading to inhibition of cell division, an observation which has often been made within routine preparation of leukaemic samples (unpublished observation). There was no association between cytogenetic failure and survival either overall (Figure 1C) or within different age groups for INT or NI patients. The OS rates for children, young adults and older adults treated intensively with successful and failed cytogenetics was: 38% v 37%, 1.04 (0.95-1.13), p=0.4; 45% v 48%,

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 Swedish studies (3, 4) concluded that cytogenetic failure was associated with an inferior outcome.

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

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

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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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
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136 Lucy Chilton[1], Christine J Harrison[1], Iona Ashworth[2], Daniel Murdy[1], Alan K Burnett[3],
137 David Grimwade[4], Anthony V Moorman[1,6], Robert K Hills[5]

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139 [1] Leukaemia Research Cytogenetics Group, Northern Institute for Cancer Research, Newcastle
140 University

141 [2] Cardiff University School of Medicine, Cardiff

142 [3] Department of Haematology, Cardiff University School of Medicine, Cardiff

143 [4] Department of Medical and Molecular Genetics, King's College London, London

144 [5] Centre for Trials Research, Cardiff University, Cardiff UK.

145 [6] Correspondence: anthony.moorman@ncl.ac.uk

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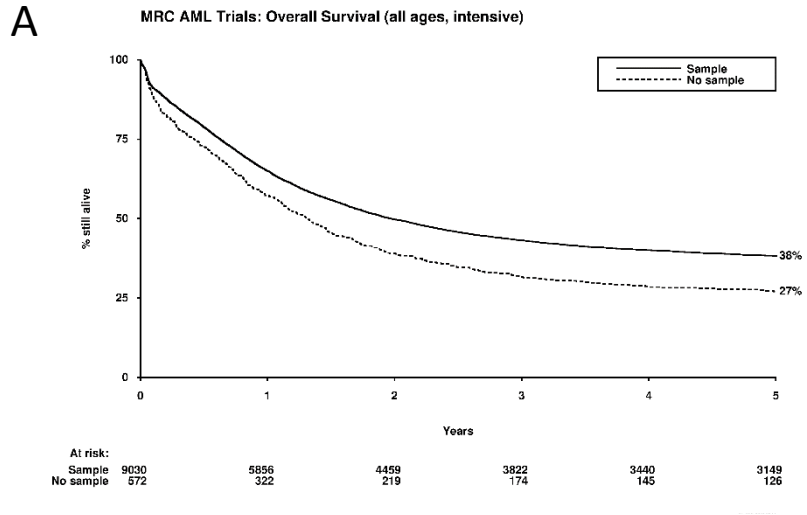
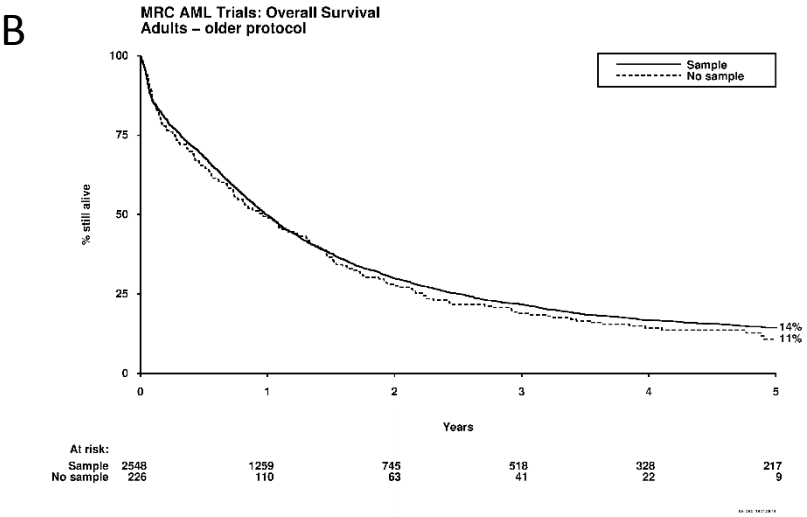
Table and Figure Legends

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

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.





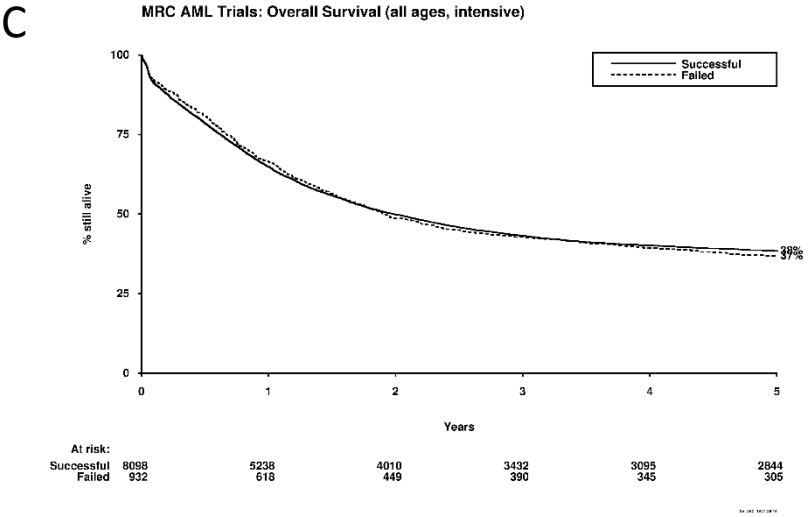


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Table 1: Demographics and clinical features for 10,685 patients treated on consecutive UK MRC acute myeloid leukaemia [1]

		Intensively treated patients						Non-intensively treated patients					
		No sample	Sample	*p*	Successful	Failed	*p*	No sample	Sample	*p*	Successful	Failed	*p*
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