

Bone Marrow Cytogenetics Workup: Application of Lean Management System to Determine if Additional Cell Workup is Helpful and Necessary to Analysis

Alvin S T Lim,¹ PhD, MHGSA, CG(ASCP), Ting Jie Chen,² Tse Hui Lim,¹ BSc(hons), MSc, CG(ASCP), Mary Tan,¹ Lai Ching Lau,¹ BSc, Ping Lim,¹ BSc, Geok Yee Lee,¹ BSc, CG(ASCP), Li Eng Loo,¹ BSc, CG(ASCP), Fiona P S Liaw,¹ BSc, Charles T H Chuah,³ M Med (Int Med), MD, MRCP, Yeow Tee Goh,³ M Med (Int Med), Sim Leng Tien,^{1,3} M Med (Int Med), FRCPA, MBA

Abstract

Introduction: High workload volumes in a Cytogenetics laboratory can lead to long result turn-around times (TAT). This study aimed to improve laboratory efficiency by adopting Lean Management System initiatives to increase productivity through the elimination of wastes. This study examined if the prerequisite 20-cell analysis was sufficient for a conclusive result or if additional cell workup was necessary to ascertain the presence of a previous chromosome abnormality among cases on follow-up, or when a single abnormal cell was encountered during the analysis to determine the presence of a clone. **Materials and Methods:** The karyotype results of cases that had additional workup were retrieved from among 8040 bone marrow cases of various haematological disorders performed between June 2003 and June 2008. **Results:** Of 8040 cases analysed, 2915 cases (36.3%) had additional cell workup. Only 49 cases (1.7%) led to the establishment of a clone. The majority of these cases could have been resolved without the additional workup, especially if fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR)-based assays had been utilised. **Conclusion:** This study shows that the additional workup procedure is redundant. The time saved by discontinuing the workup procedure can be used to analyse other cases, leading to increased laboratory efficiency and a faster TAT without compromise to patient care. The practice of additional workup over and above the 20-cell analysis should be dispensed with as little benefit was derived for the amount of additional manpower expended. FISH or PCR-based assays should be utilised to elucidate a case further.

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Introduction

Cytogenetic studies are important to the diagnosis and monitoring of various haematopoietic disorders such as myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), lymphoproliferative disease (LPD), and multiple myeloma (MM). It can also provide a prognosis to a patient's response to certain treatment regimes. For example, deletion 5q is one of the most frequent chromosomal abnormalities seen in MDS. A positive cytogenetic finding

of this lesion indicates a favourable prognosis with longer median survival.^{1,2} In contrast, monosomy 7 or deletion 7q, a common abnormality in MDS, is associated with a poor response to chemotherapy and there is frequent progression to acute leukemia within a short period of time.³⁻⁵

Apart from accurate diagnoses, a good Cytogenetics laboratory needs to report the result within the shortest turn-around time (TAT) possible. This is desirable for improved patient management. Due to the high volume of haematological samples being processed in our laboratory, the TAT to finalise a bone marrow result is about 2 weeks or

¹ Department of Pathology, Singapore General Hospital

² Temasek Applied Science School, Temasek Polytechnic

³ Department of Haematology, Singapore General Hospital

Address for Correspondence: Dr Alvin Lim Soon Tiong, Cytogenetics Laboratory, Department of Pathology, Singapore General Hospital, Outram Road, Singapore 169608.

Email: alvin.lim.s.t@sgh.com.sg

even 3 weeks if there is an increased backlog of cases due to an unexpected avalanche of referral cases compounded by staff on long leave.

Laboratory efficiency may be improved with the application of the Lean Management System to increase productivity through the elimination of wastes. Seven types of wastes have been identified; (i) transportation (moving products that are not required to perform the processing), (ii) motion (people or equipment moving/ walking more than required to perform the processing), (iii) inventory (all components, work-in-progress and finished product not being processed), (iv) waiting (waiting for the next production step), (v) overproduction (production ahead of demand), (vi) over processing (doing more than is required within a process) and (vii) defects (effort involved in inspecting and fixing defects).⁶ Laboratory efficiency can also be improved with the increased use of laboratory instrumentation to automate routine bench work procedures or modifying an existing protocol.⁷

One particular “Kaizen” (change for the better) we have identified was whether we could streamline the current analysis process which was thought to be a potential case of over processing.

To address this issue, we determined if our routine 20- or 30-cell analysis alone was sufficient to make a firm diagnosis or if there was still a need for a further confirmatory check with additional cell workup. Dispensing with the practice of extra workup will save time that can be used to analyse other cases, resulting in a faster result TAT, providing that patient care is not compromised. To evaluate this, results from archival cases performed at the Cytogenetics Laboratory in Singapore General Hospital from June 2003 to June 2008 were retrieved and analysed. These included cases that were in remission that had additional cell workup done and diagnostic cases that were found with single abnormal cells (SAC) that had additional cells checked.

The College of American Pathologists (CAP) and American College of Medical Genetics (ACMG) have established minimum criteria for the numbers of cells to be counted, analysed and karyogrammed for various tissue types and culture methods. The CAP Cytogenetics checklists (2007) and the ACMG Standards and Guidelines for Clinical Cytogenetics Laboratories (2006) require 20 fully analysed cells as the minimum prerequisite for all bone marrow studies.^{8,9}

However, no guidelines are given with regard to the number of additional cells that need to be scored in the event an SAC is encountered during the course of a study, or the number of additional cells that need to be checked for a previous abnormality in cases on follow-up. This is left to the discretion of the laboratory director. By definition, an abnormal clone is defined as at least 2 cells having the same

extra chromosome or a structurally rearranged chromosome, or at least 3 cells having lost the same chromosome within a chromosome study.^{10,11} Cells having fewer than the above required numbers of cells are defined as SACs.

Materials and Methods

Patients

Results were retrieved of bone marrow analyses (including bone core and blood) performed between June 2003 and June 2008 at the Singapore General Hospital's Cytogenetics Laboratory, Department of Pathology.

Culture Set up

All specimens were set up as 2 independent cultures each, one for direct harvest and the other for a 24-hour, 48-hour or 72-hour culture, depending on the disease type. The direct harvest culture was set up in Direct Harvest medium containing pre-warmed KCl hypotonic, Colcemid™, EDTA and trypsin. The other culture contained 10 mL culture medium (RPMI 1640 supplemented with Chang medium, Irvine, USA). Bone core specimens were cut into small pieces using sterile scissors and set up as per bone marrow specimen.

Harvest and Slide-making Procedures

All cultures were incubated in the Direct Harvest medium at 37°C for 30 minutes and then passed through a series of fixative steps until the supernatant became clear. The cell pellets were re-suspended and placed in a refrigerator until slide-making day. The fixative was changed just before slide-making. The cell suspensions were dropped onto cold, wet pre-cleaned slides and then placed overnight on a 60°C slide warmer and in a 90°C oven the next day.

Chromosome banding was carried out using standard GTG banding method. After staining, the slides were dried and mounted with DPX mounting medium and coverslipped.

Chromosomal Analysis

Microscopic analysis was performed under 1000 X magnification (Olympus, Tokyo, Japan) and appropriate metaphases were captured and karyogrammed with the Powergene MacKtype™ version 5.6 software (Applied Imaging, UK). Twenty to 30 metaphases were fully analysed, split between the 2 cultures. Five metaphases were karyogrammed with each diagnostic case and between 2 and 4 metaphases for follow-up cases. All karyotype designations were made in accordance with the International System of Cytogenetic Nomenclature.^{10,11}

Laboratory Guidelines for Analysis

Up to 2007, for diagnostic cases (new cases) of all haematological disorders, 30 fully analysed metaphases were required, where possible. If an SAC was encountered, a

further 10 cells were checked for the presence of a clone. For follow-up cases, including cases in remission or at relapse, 30 fully analysed metaphases were required, where possible, and an additional 20 cells were checked for the presence of a previous abnormality. If an SAC was encountered, another 10 cells were checked for clonality. From 2007, the number of fully analysed metaphases required was revised to 20, in keeping with CAP and ACMG guidelines. The numbers of additional cell checks remained unchanged.

Fluorescence in Situ Hybridisation (FISH)

FISH was performed on selected cases as ordered by the referring doctor. The probes used comprised dual colour dual fusion probes, break-apart probes and locus specific probes, with appropriate controls (Abbott Molecular, USA). All procedures were carried out in accordance with the manufacturer's recommendations.

Results

Between June 2003 and June 2008, a total of 8040 bone marrow cases, comprising 5005 diagnostic cases (62.3%) and 3035 follow-up cases (37.7%), were performed at the SGH Cytogenetics Laboratory. These were sub-divided into 'No results', 'Normal karyotype', and 'Abnormal karyotype'. Among the follow-up cases, the number of cases with additional cells scored for those with normal and abnormal karyotype were 1160 cases and 221 cases, respectively (total = 1381 cases). Among the diagnostic cases, the number of cases with additional cells scored for those with normal and abnormal karyotype were 1297 cases and 237 cases, respectively (total = 1534 cases). Therefore, there were altogether 2915 cases (36.3%) that had additional workup. These sub-divisions were further classified as 'with informative findings' (a clone was found) and 'without informative findings' (no clone was found). In total, there were only 49 cases out of 2915 cases (1.7%) that yielded informative findings. The schematic representation of these sub-divisions is presented in Figure 1.

Table 1 lists the cytogenetic findings of these 49 cases with additional cell workup which resulted in an abnormal clone being found. Five of these cases, cases 15, 32, 33, 46 and 47, are discussed (Tables 2 to 6).

Figure 2 shows the total number of bone marrow cases per year from June 2003 to June 2008 (7 months in 2003 and 6 months in 2008) and the number and percentage of cases that had additional cell workup for that year. This ranged from 29.7% in 2008 to 42% in 2006 (mean = 35.7%).

Discussion

In our laboratory, the average time to complete the prerequisite 20-cell analysis of a bone marrow case is about 2 hours 40 minutes. The average time spent looking up an additional 20 cells for a chromosome

abnormality is 55 minutes. Therefore, the total time spent on a case with additional cell workup is about 3 hours 35 minutes. This additional time spent is about 34% of the total time spent on a typical case. Consequently, a longer analysis time may delay a result which already takes about 2 weeks on average.

Additional cell workup procedures have a negative impact on the result TAT by taking up additional technologist time that could have been used to analyse other cases. Such over processing also results in an increase in backlogged cases. Of the 8040 cases processed between June 2003 and June 2008, it was found that an amazing 36.3% (2915 cases) had additional cell workup. However, only 49 cases resulted in the finding of an abnormal clone. Therefore, the success rate of finding an abnormal clone with additional work was an abysmal of 1.7%. In contrast, the total time spent on the additional workup of the 2915 cases was calculated to be about 2672 hours, based on an additional 20-cell workup per case. This amount of time could have been used to analyse another 746 cases, thereby effectively clearing up many of the backlogged cases. The percentage of cases with additional cell workup had been fairly consistent between 2003 and 2005 (36.2% to 38.8%) before hitting a high of 42% in 2006. However, it began to tail off from 2007 (30.9%) when the issue of possible excessive work was first surfaced and some measures were taken to reduce additional cell counts, coupled with a reduction from 30 fully analysed cells to just 20 cells, in keeping with CAP and ACMG guidelines (Fig. 2).^{8,9}

The additional cell workup protocol has proved to be ineffective and has contributed to a prolonged result TAT. Furthermore, of the 49 cases that were informative, the majority could have been resolved without further cell workup (Table 1). FISH or PCR-based assays could have been utilised to resolve a number of these cases.

A few cases are highlighted for discussion in support of discontinuing the practise of additional cell workup.

The first case, Case 15 (Table 2), was a patient with ALL who had an unbalanced karyotype with a der(22)t(9;22) Philadelphia (Ph) chromosome as the sole abnormality from a previous chromosome study. The current investigation did not reveal any chromosome abnormalities in the first 20 cells analysed but a single cell with the Ph chromosome was found only after 30 additional cells were scored. Bone marrow and other clinical findings had indicated that the patient was in remission. Retrospectively, the additional workup could have been more effectively replaced by qualitative fluorescent polymerase chain reaction (QF-PCR) or interphase FISH using a BCR/ABL1 dual colour dual fusion probe coupled with an internal control such as a centromeric probe for chromosome 9 in a third colour. Instead of expending 82 minutes screening an additional

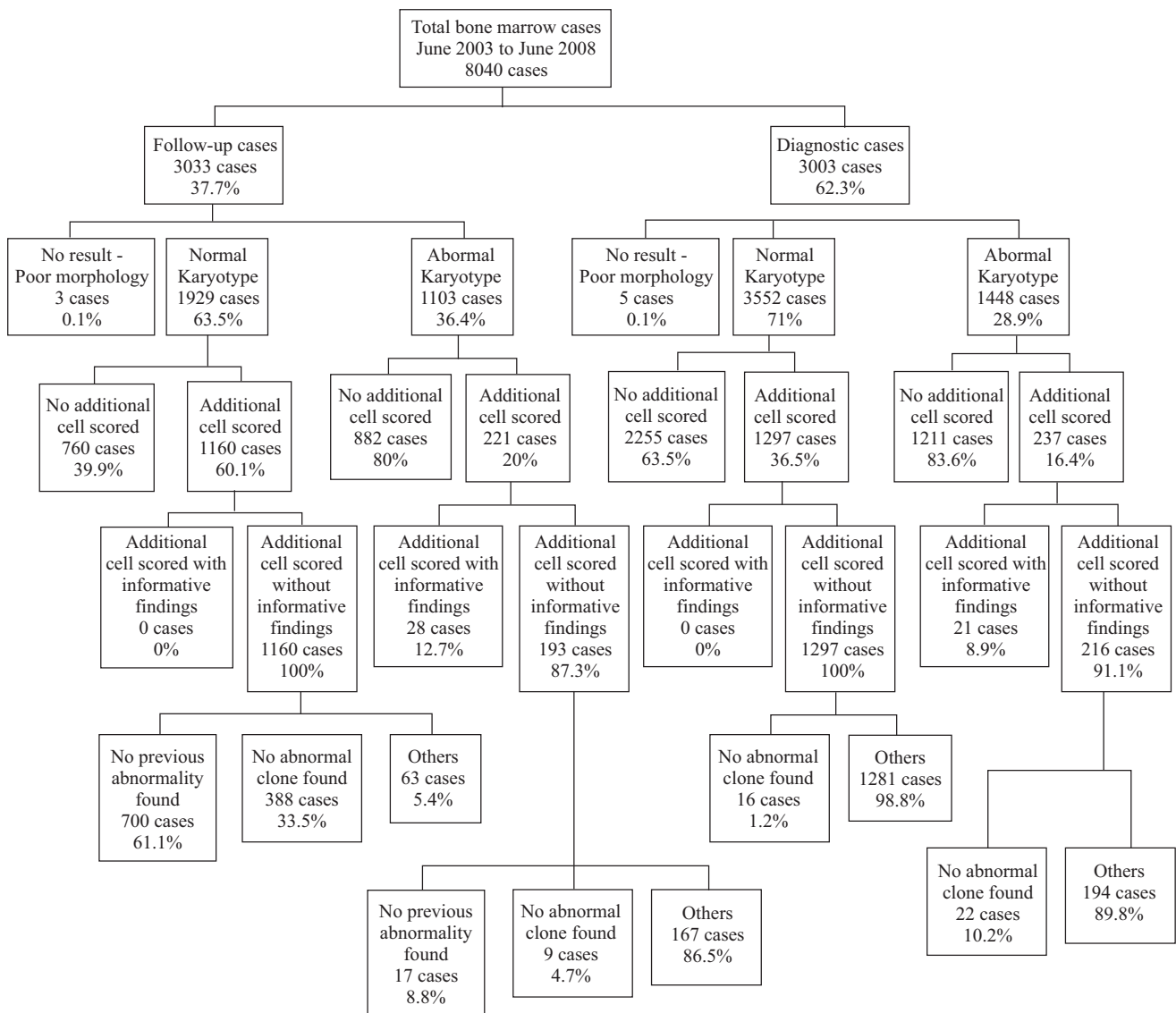


Fig. 1 Schematic representation of bone marrow sub-division classification.

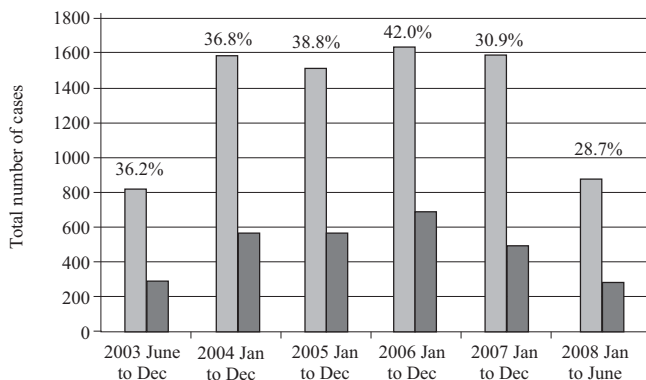


Fig. 2 Number of cases with additional cell workup between June 2003 and June 2008.

30 cells, FISH would have accomplished the task within a few minutes.

Case 32 was diagnosed with thrombocytopenia (Table 3). An abnormal clone comprising an interstitial deletion of 20q was present in the 20 out of 21 cells analysed. A single cell with an extra copy of the deleted chromosome 20q was found within the first 20-cell analysis but the second clone was found only after a further 35 additional cells were scored. Retrospectively, since an abnormal clone had already been found, it was unnecessary to hunt down the second clone since the management of the patient would not have been different with the finding of this second abnormal clone. The time spent on the 35 additional cells was more than 1½ hours of the technologist time.

Case 33 was a patient with non-marginal zone lymphoma (MZL) (Table 4). An SAC with an interstitial deletion of the long-arm of chromosome 7, del(7)(q22q36), was found

Table 1. Karyotype Findings of Patients with Additional Cells Scored that Resulted in a Clone Being Found

Case No.	Disease diagnosis	Karyotype	No. additional cells scored
1	CML on Glivec®	47,XX,+8[14]/46,XX,der(9)t(9;22)(q34;q11.2)ins(9;7)(q34;q31q31),der(22)t(9;22)[1]/46,XX[6]	30
2	MM	41~42,X,-X,add(1)(p13),der(2)t(2;7)(p13;q11.2),add(3)(q27),add(5)(q33),del(6)(q13),-13,-17,-22,+1~5mar[cp3]/46,XX[19]	12
3	CML on Glivec®-AcceleratedPhase	46,XX,t(9;22)(q34;q11.2)[19] 68,XX,+X,+1,+1,+2,+4,+6,+8,t(9;22)(q34;q11.2),+10,+11,+13,+14,+15,+15,+17,+18,+19,+19,+20,+21,+21,+der(22)t(9;22)x2[2]	20
4	Pancytopenia	46,XY,del(16)(q22)[2]/46,XY[28]	10
5	MM	51,XY,+4,+7,der(7;17)(q10;q10),+9,t(11;14)(q13;q32),+der(14)t(11;14)(q13;q32),+15,+19[2]/46,XY[28]	10
6	CLL	46,XY,del(20)(q11.2)[2]/46,XY[18]	30
7	MM	42,X,-Y,i(1)(q10),add(3)(p11.1),add(6)(q25),-8,add(10)(p11.1),-13,-16,-16,-22,+1~2mar[cp3]/46,XY[22]	15
8	MM	42,XY,add(1)(p13),+add(1)(p13),-2,-2,-6,-13,-14,add(14)(q22),-16,-19,+mar1,+mar2[cp2]/46,XY[28]	50
9	AML	46,XX,t(6;12)(q21;q24.1)[5]/46,XX,t(5;21)(q13;q22)[2]/46,XX[14]	30
10	Lymphoma	50~56,XY,+Y,add(1)(q32),add(5)(q31),+del(6)(q21q25),del(13)(q31q34),add(19)(q13.4),+21,+21,+mar1x3,+mar2,+mar3[cp4]/46,XY[20]	12
11	ALL	45~46,XX,-3,add(5)(q35),-7,add(17)(p12),-19,+mar1,+mar2,+mar3[cp3]/46,XX[19]	30
12	Bicytopenia	48,XY,dic(2;6)(q34;q11),-9,del(11)(p14),der(12)del(12)(p13)add(12)(q21),+13,add(15)(q11.2),+der(15)add(15)(p11.1)add(15)(q22),der(18)t(11;18)(q13;q23),+der(?)t(?)11(?)q13[2]/46,XY[18]	10
13	ALL	46,XY,add(5)(q13),add(7)(p15)[2]/46,XY[20]	10
14	ALL	45,X,-Y,add(6)(q13) or del(6)(q13q23)[2]/46,XY[29]	2
15	ALL	46,XX,der(22)t(9;22)(q34;q11.2)[1]/46,XX[20]	30
16	MM	46,XY,del(20)(q11.2)[2]/46,XY[19]	10
17	AML	45,X,-Y,t(6;21)(p21.1;q22)[16]/45,idem,+der(1)add(1)(p11)del(1)(q31q31),der(1;7)(p10;p10)[3]/45,idem,add(7)(q11.2)[2]	5
18	CML	47,XX,+8,t(9;22)(q34;q11.2)[1]/47,XX,+8[1]/46,XY[20]	12
19	MDS	47,XY,+add(9)(p11)[2]/46,XY,del(17)(p13)[2]/46,XY[18]	13
20	CLL	46,X,add(Y)(p11.2),del(1)(p34.1p35),add(6)(p22),add(17)(p13)[2]/46,XY[29]	21
21	MDS	47,XX,+8[3]/46,XX[19]	5
22	MDS	47~48,XY,+Y,+mar[cp5]/46,XY[19]	10
23	MM	47,X,-X,add(6)(q11),add(10)(p13),+mar1,+mar2[2]/46,XX[17]	8
24	MM	52~57,XX,+X,+X,+8,+12,+13,+22[cp3]/46,XX[19]	10
25	MPD	48,XX,+8,+9[2]/46,XX[19]	5
26	CML	46,XY,del(11)(q21),add(12)(p11.2),add(18)(p11.2)[cp3]/46,XY[19]	10
27	MPD	46,XY,der(10)t(1;10)(q12;p15)[6]/46,XY,+1,der(1;22)(q10;q10)[3]/46,XY,+1,der(1;14)(q10;q10)[2]/46,XY[11]	10
28	CML	46,XY,t(9;22)(q34;q11.2)[1]/46,XY[19]	17
29	MDS	47,XY,+Y[2]/46,XY[19]	10
30	CML on Glivec®	46,XY,t(2;9;22)(q35;q34;q11.2)[16]/46~47,idem,+8,i(17)(q10)[cp10]/46,XY[2]	10
31	PRV	46,XY,add(21)(q11.2)[3]/46,XY[18]	50
32	Thrombo-cytopenia	46,XY,del(20)(q11.2q13.1)[18]/47,idem,+del(20)[2]/46,XY[1]	35
33	Lymphoma	46,XY,del(7)(q22q36)[2]/46,XY[20]	20
34	CML	46,XY,t(9;22)(q34;q11.2)[18]/46,idem,add(2)(q31),inv(3)(q21q26)[2]	10
35	CML-Chronic phase	46,XY,t(2;9;22)(q37;q34;q11.2)[1]/46,XY[20]	10

Table 1. Contd.

Case No.	Disease diagnosis	Karyotype	No. additional cells scored
36	CML	46,XY,t(9;22;19)(q34;q11.2;q13.2)[19]/47,idem,+8[2]	8
37	Lymphoma	46,XX,t(7;14)(q36;q11.2)[2]/46,XX[19]	10
38	CML-Accelerated Phase	46,XY,t(9;22)(q34;q11.2)[18]/47,idem,+8,i(17)(q10)[3]	30
39	MM	56~61,XY,+X,+der(1;16)(q10;p10)x2,der(2)t(2;6)(q33;p11),+der(2)t(2;6)(q33;p11),+3,+add(3)(q21),+7,+del(8)(q24.1)x2,+9,-11,-13,der(14)t(11;14)(q13;q32)dup(11)(q13q22),+16,+21,+21[cp3]/46,XY[18]	33
40	Lymphoma	47,XX,+3[3]/46,XX[19]	10
41	CML onDasatinib	46,XY,t(2;9;22)(p23;q34;q11.2)[1]/46,XY[20]	20
42	MDS	46,XY,i(14)(q10)[2]/46,XY[19]	4
43	CML	46,XY,add(20)(q11.2)[3]/46,XY[19]	20
44	Lymphoma	50,XY,+add(1)(p13),add(2)(q37),+6,+2mar[2]/46,XY[19]	35
45	CML-ChronicPhase	47,XX,+8[21]/46,XX,t(9;22)(q34;q11.2)[2]	10
46	MM	42,X,-X,del(1)(p22p32),der(2)t(1;2)(q21;p25),t(8;13)(q24.1;q11),del(12)(p11.1),-13,-21,-22[1]/46,XX[20]	7
47	CML onGlivec®	47,XX,+8[6]/46,XX,t(9;22)(q34;q11.2)[5]/47,XX,+6[2]/46,XX[8]	10
48	CLL	45~46,XY,+add(10)(q22),del(12)(q24.1),add(14)(q22),add(16)(q23),add(17)(p11.1),-19,del(20)(q11.2)[cp3]/46,XY[18]	7
49	MM	52,X,-Y,+add(1)(p13)x2,+2,add(3)(p21),del(4)(p12p16),der(4;8)(q10;q10),+add(5)(q14),+add(6)(q13),+7,+9,+15,add(17)(p11.2)[6]/104,idemX2[2]/46,XY,+1,der(1;7)(q10;q10)[1]/46,XY[12]	5

ALL: Acute Lymphoblastic Leukaemia; CLL: Chronic Lymphocytic Leukaemia; CML: Chronic Myelogenous Leukaemia; MDS: Myelodysplastic Syndrome; MM: Multiple Myeloma; MPD: Myeloproliferative Disorder

Table 2. Clinical Findings and Analysis of Case no. 15

Case No. 15	
Specimen	Follow-up
Sex/Age	Female / 42
Clinical diagnosis	Acute lymphoblastic leukaemia
Bone Marrow finding	Mild erythroid hyperplasia. No morphological evidence of leukaemia
Cells analysed	21
Additional cells scored	30
Karyotype	46,XX,der(22)t(9;22)(q34;q11.2)[1]/46,XX[20]
Cytogenetic result	1 metaphase out of 20 analysed and 30 cells scored had a Ph chromosome. This single chromosomal abnormality was seen in an earlier sample dated 21/12/04.
Comment on additional cells scored	Found abnormal cell at the 30th cell scored. Case is in remission. FISH could have been done to score for the Ph chromosome.

within the first 20-cell analysis. A clone was established when a second cell with del7q was found following an additional 20-cell workup. Since del7q is closely associated with MZL and certain non-MZL including follicular lym-

phomas and mantle cell lymphomas,¹²⁻¹⁴ albeit to a lesser extent, the single abnormal cell found could have been reported. Because of the low level of abnormal cells, FISH using the D7S486 probe (Abbott Molecular, USA) could also have been performed to confirm the abnormality instead of utilising the additional 50 minutes of technologist time which may not have resulted in the establishment of a clone.

With case 46, a patient with multiple myeloma (MM), a previous cytogenetic investigation showed a hypodiploid karyotype that included structural aberrations. A follow-up investigation did not reveal any abnormal cell in the 20-cell analysis but an abnormal cell with the same previous abnormalities was subsequently found after 7 additional cells were scored (Table 5). The patient was not in remission as her bone marrow aspirate showed 5% plasma cells, consistent with persistence of MM. An adjunct FISH test done earlier had detected a clone (8%) with deletion of RB1 and fusion signals of FGFR3/IGH. The abnormal karyotype was probably the same clone as that detected by FISH but owing to the limitation of cytogenetic band resolution of ~5 Mb,¹⁵ the FGFR3/IGH fusion arising from a t(4;14) rearrangement, and sometimes deletion of 13q14, was undetectable.

Table 3. Clinical Findings and Analysis of Case no. 32

Case No. 32	
Specimen	Diagnostic
Sex/Age	Male / 92
Clinical diagnosis	Thrombocytopenia
Bone Marrow finding	Erythroid hyperplasia. Megakaryocytes seen with mild dysplasia.
Cells analysed	21
Additional cells scored	35
Karyotype	46,XY,del(20)(q11.2q13.1)[18]/47,idem,+del(20)[2]/46,XY[1]
Cytogenetic result	20 out of 21 cells analysed had an interstitial deletion of the 20q. Two of these cells had an additional copy of the del 20q. Deletion of 20q is associated with MDS, AML and MPD.
Comment on additional cells scored	2 nd cell of the 2 nd abnormal clone was found on the 35 th cell scored.

Table 4. Clinical Findings and Analysis of Case no. 33

Case No. 33	
Specimen	Follow-up
Sex/Age	Male / 33
Clinical diagnosis	Non-marginal zone lymphoma
Bone Marrow finding	Bone marrow aspirate shows erythroid and megakaryocytic hyperplasia with plasmacytosis. Few atypical lymphoid cells and increased numbers of malt cells.
Cells analysed	22
Additional cells scored	20
Karyotype	46,XY,del(7)(q22q36)[cp2]/46,XY[20]
Cytogenetic result	2 out of 22 metaphases analysed had an interstitial deletion of 7q. This is a new abnormality. Previous abnormalities were not seen.
Comment on additional cells scored	2 nd cell of the abnormal clone found on the 20 th cell scored. FISH could have been done to score for del7q.

Table 5. Clinical Findings and Analysis of Case no. 46

Case No. 46	
Specimen	Follow-up
Sex/ Age	Female / 66
Clinical diagnosis	Multiple Myeloma
Bone Marrow Finding	Bone marrow aspirate shows mild megakaryocytic hyperplasia with 5% plasma cells present.
Cells analysed	21
Additional cells scored	7
Karyotype	42,X,-X,del(1)(p22p32),der(2)t(1;2)(q21;p25),t(8;13)(q24.1;q11),del(12)(p11.1),-13,-21,-22[1]/46,XX[20]
Cytogenetic result persistence	Previous abnormalities were seen in 1 cell out of 21 cells analysed. The result is consistent with of the disease.
Comment on additional cells scored	Previous abnormality was not found on the 20 cells analysis but found on the 7th additional cell scored. Case was not in remission.
FISH test	
MM panel probe	1. LSI FGFR3 (4p16.3)/IGH@(14q32) DF2. LSI CCND1(11q13)/IGH@(14q32) DF3. LSI RB1(13q14)4. LSI TP53(17p13.1)
FISH result	8% of 200 nuclei showed fusion signals for FGFR3/IGH@.5.7% of 400 nuclei showed loss of one copy of RB1 gene. The test is positive for MM FISH panel.

Table 6. Clinical Findings and Analysis of Case no.47

Case No. 47	
Specimen	Diagnostic
Sex/Age	Female / 41
Clinical diagnosis	Chronic myeloid leukaemia (CML) on Glivec®
Cells analysed	21
Additional cells scored	10
Karyotype	47,XX,+8[6]/46,XX,t(9;22)(q34;q11.2)[cp5]/47,XX,+6[2]/46,XX[8]
Cytogenetic result	A Ph chromosome from a translocation between chromosomes 9 and 22 was present in 5 out of 21 cells analysed. Two other cell lines, trisomy 8 and trisomy 6, were also present in this sample. The presence of a Ph negative trisomy 8 clone may be imatinib therapy-induced. The significance of the trisomy 6 clone is unclear.
Comment on additional cells scored	Found 3rd abnormal clone after the 10th cell scored. However, 2 abnormal clones were already seen in the routine 20-cell analysis. The 3rd abnormal clone may be treatment related.

Case 47 (Table 6) was a CML case on Glivec®. An abnormal 47,XX,+8[6]/46,XX,t(9;22)(q34;q11.2)[cp5]/47,XX,+6[2]/46,XX[8] karyotype with 3 abnormal clones was found. The first 2 clones, t(9;22) and trisomy 8, were found within the 20-cell analysis. The third clone, trisomy 6, was found only after 10 additional cells were scored. Trisomy 8 without a Ph chromosome is known to be imatinib treatment-related and is not indicative of disease progression. Trisomy 6 without a Ph chromosome may likewise be treatment-related although there has been no report of this in the literature. Since the significance of trisomy 6 is unknown, the management of the patient was unaltered. We suggest that with such cases, a comment be made in the results to indicate that a single cell with trisomy 6 was found, instead of having to perform additional cell workup to determine the presence of a clone.

For follow-up cases for disease monitoring where the gene rearrangements are very specific to the disease, such as BCR/ABL1-positives in CML, AML1/ETO-positives in AML-M2, or PML/RARA-positives in AML-M3, it is highly recommended that in place of an additional cell workup procedure a FISH or PCR-based test such as QF-PCR be performed, where possible. These assays are far more sensitive and will be able to detect very low level minimal residual disease of 2 to 3 or cells or fewer per hundred with FISH to just 1 cell in several thousands with PCR. With this strategy, it is likely that the detection rate would have been far more than the 1.7% obtained by additional cell workup in the 2915 cases. Moreover, monitoring of a cryptic chromosomal rearrangement such as a masked Ph can only be achieved with FISH or QF-PCR. In the case of an SAC, a remark could be made in the report about the abnormal cell and adopt a watchful waiting approach. In addition, if an abnormality is disease-specific, e.g. a Ph chromosome seen in a case of CML, only 1 cell may be sufficient for reporting, especially with compelling evidence from FISH

or other clinical findings.¹⁶

Conclusion

Adopting Lean initiatives at the Cytogenetics laboratory will lead to improved efficiencies by reducing wastage. This study investigated whether the additional cell workup procedure was effective among haematological disorders in follow-up of cases with a previous abnormality and in diagnostic cases presenting single cell abnormalities. It was concluded that this practice should be dispensed with as little benefit was derived for the amount of additional manpower expended. The prerequisite 20-cell analysis is fully adequate. If warranted, FISH or PCR-based assays should be utilised to further elucidate a case.

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