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Challenges of Pre-analytical Variables in Conventional Cytogenetics - A University Teaching Hospital Experience

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ABSTRACT

Introduction: Conventional Cytogenetics is an essential tool for the diagnosis of a neoplastic or premalignant condition and provides important prognostic and therapeutic information. The rate of unsuccessful karyotyping in hematological malignancies is reported between 10-20%.

Objective: To analyze the relationship between pre-analytical variables and associated failures with conventional karyotyping in hematological neoplasms.

Material and Methods: 1020 samples with suspected hematological malignancies received in the department of Cytogenetics from January 2018 to December 2019 were included in the study. Pre-analytical variables assessed included time from collection to sample processing, type of sample, diagnosis and sample cellularity. Statistical analysis was performed using Chi-square test to verify associations of variables with karyotyping.

Results: 86 (12%) out of 720 samples that were processed in less than 24 hours of time of collection showed an unsuccessful KT while 57/300 samples (19%) that were processed beyond 24 hours failed to yield any metaphase (p-value 0.003). 31/79 PVB (39%) and 112/941 BM (12%) were unsuccessful (p-value <0.001). 15/287 (06%) acute myeloid leukemia cases, 64/156 (41%) acute lymphoblastic leukemia cases, 12/109 (11%) myelodysplastic syndrome, 6/34 (18%) MDS/MPN cases, 11/338 (03%) MPN cases and 35/96 (36%) from the others category did not yield any metaphase for analysis (p value 0.015). 24/819 (03%) samples with cellularity >7 x 10³/μl and 119/201 (59%) samples with low cellularity being ≤7 x 10³/μl were unsuccessful (p-value <.001).

Discussion: Rate of unsuccessful karyotyping was 14% that was significantly associated with the time to process, nature of sample and cellularity.

Conclusion: Collection of an adequate and good quality sample is of paramount importance on which success of karyotyping of hematological malignancies depends.

Key Words: Pre-analytical variables, Karyotyping, Cytogenetics, Hematological malignancies

INTRODUCTION

The World Health Organization (WHO) classification of tumors of the hematopoietic and lymphoid tissues incorporates cytogenetic and molecular genetics abnormalities.¹

Hematologic neoplasm is listed in the top ten malignancies worldwide and also one of the leading causes of mortality in patients with cancer. Almost 9% of all cancer cases diagnosed in a year are hematological malignancies.^{2,3} Identification of clonal aberrations yields support in diagnosing malignant/premalignant diseases as well as gives important information regarding prognosis and therapy.^{4,5} Therefore,

conventional cytogenetic analysis is mandatory in the evaluation of suspected acute leukemia. It is one of the essential tools for classification of hematological malignancies, prognostication and treatment.^{6,7} It is also seen that unsuccessful conventional karyotyping has a prognostic implication in hematological malignancies.^{8,9,10}

The rate of unsuccessful karyotyping in hematological malignancies is reported between 10-20%. Successful karyotyping is affected by number of factors like nature of sample, time to process, collection method, cellularity of sample and processing methods.¹¹ The present study aims to analyze the

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relationship between pre-analytical variables and associated failures with conventional karyotyping in hematological neoplasms.

MATERIAL AND METHODS

The archives of department of Cytogenetics were retrospectively reviewed from January 2018 to December 2019. Of the 4300 cytogenetic case records reviewed over a period of 2 years, 1020 samples of bone marrow and blood with suspected hematological malignancies were received over a period of 2 years. Along with clinical history and examination, peripheral blood counts, marrow morphology and immunophenotyping was done to come to a diagnosis. Molecular tests like JAK2 mutation, Ph chromosome etc. were done, wherever required. Final diagnosis was made based on WHO criteria.¹ Bone marrow samples were cultured for 17 and 24 hours without mitogenic agents and harvested following standard protocols.⁷ The slides were air-dried and stained with G-Banding using Trypsin and Giemsa (GTG-banding). Twenty metaphases were analyzed and karyotypes were described according to the International System for Human Cytogenetic Nomenclature criteria.¹²

Pre-analytical parameters

The following pre-analytical variables were noted:

1. Time from sample collection to initiation of processing in lab (more or less than 24 hours)
2. Material type (bone marrow or peripheral venous blood)
3. Sample cellularity (>7 or $\leq 7 \times 10^3/\mu\text{l}$)
4. Patient's diagnosis (AML, ALL, MDS, MPN, CLL, MDS/MPN)

Statistical analysis was performed using Chi-square test to verify associations of variables with karyotyping. The data were analyzed using IBM SPSS software (Version 21). The level of significance for the statistical tests was 5% (p-value < 0.05).

RESULTS

Out of the 4300 cases received in Cytogenetics lab over a period of 2 years, 1020 samples with hematological malignancies were found. The mean patient age was 46 years (range 1 month – 84 years). There were 612 (60%) males and 408 (40%) females with a male : female ratio of 1.5. The diagnosis of 1020 cases as per the WHO criteria was as follows: myeloproliferative syndrome 338 (33%), acute myeloid leukemia 287 (28%), acute lymphoid leukemia 156 (15%), myelodysplastic syndrome 109 (11%), MDS/MPN 34 (03%) and Others like plasma cell disorder, chronic lymphoid leukemia etc 96 (09%).

Out of the 1020 samples, there were 941 (92%) bone marrow (BM) specimen and 79 (08%) peripheral venous blood (PVB) specimens. 720 (71%) samples had culture set up in less than 24 hours from their time of collection. 819 (80%) samples had cellularity more than $7 \times 10^3/\mu\text{l}$.

143 (14%) samples out of 1020 failed to yield any metaphase on culture that precluded any cytogenetic analysis. The pre-analytical variables associated with unsuccessful or failed karyotype (KT) are shown in table 1. 86 (12%) out of 720 samples that were processed in less than 24 hours of time of collection showed an unsuccessful KT while 57/300 samples (19%) that were processed beyond 24 hours failed to yield any metaphase (p-value 0.003). 31/79 PVB (39%) and 112/941 BM (12%) were unsuccessful (p-value < 0.001). 15/287 (06%) AML cases, 64/156 (41%) ALL cases, 12/109 (11%) MDS, 6/34 (18%) MDS/MPN cases, 11/338 (03%) MPN cases and 35/96 (36%) from the Others category did not yield any metaphase for analysis (p-value 0.015). 24/819 (03%) samples with cellularity $> 7 \times 10^3/\mu\text{l}$ and 119/201 (59%) samples with low cellularity being $\leq 7 \times 10^3/\mu\text{l}$ were unsuccessful (p-value < 0.001).

DISCUSSION

Cytogenetic analysis remains one of the essential tools for classification of hematological malignancies, prognostication and treatment. WHO criteria for diagnosis of hematological malignancies includes cytogenetic abnormalities. However, despite its relevance, sometimes G-banding karyotyping fails to give results due to technical difficulties that yield poor mitotic index.¹

In the present study, 143/1020 (14%) samples did not yield any metaphase for analysis and therefore were categorized as Unsuccessful karyotyping (UK). This UK's rate lies between the accepted range of UK reported in literature in case of hematological malignancy (10–20%).^{6,11} As per the guidelines, a minimum of 20 metaphases must be analyzed in the absence of any abnormality. It will help in excluding presence of 14% of any abnormal clone with 95% confidence.¹³ The remaining 877 samples, 810 (92.4%) yielded 20 metaphases, 35 samples (4%) had 10-20 which were categorized as 'Incomplete karyotype' and 32 (3.6%) had less than 10 metaphases which were labeled as 'Insufficient karyotype'.

In our lab, 88% of the samples (634/720) processed within 24 hrs of its time of collection were successful. We had a higher success rate with samples processed in less than 24 hrs (86/720, 12% UK) when compared to those that were processed after 24 hrs (57/300, 19%). Unlike the work done by Santos and his coworkers, it was statistically significant (p-value 0.003).⁶ As per the literature, it is recommended to send a sufficient quantity of BM (0.5–1 ml minimum), preferably within 24 h after aspiration (best is as soon as possi-

ble) to the Cytogenetics laboratory. Care should be taken to avoid delays in transport and exposure to extreme temperatures. Use of transport medium is also strongly recommended to minimize drying-out of the sample and to maintain the viability of the cells.^{4,14,15}

For hematological malignancies including myeloma the specimen of choice for analysis is bone marrow. Peripheral venous blood should be used only if there is a significant level of circulating disease.¹⁶ In our study, we found that unsuccessful Karyotype was seen at a higher rate in PVB (39%) when compared to BM (12%). The nature of sample - PVB and BM showed a statistically significant difference (p -value <0.001) in the outcome of metaphase yield where PVB was found to be associated with UK. This is in concordance to reports published by other authors.^{6,17} PVB may yield informative results when the circulating blast cell percentage is higher than 10% and should be considered as an alternative where a BM sample or culture has proved inadequate. It is inappropriate for all diagnoses, like MDS, MPD (except chronic granulocytic leukemia and myelofibrosis) or pancytopenic AML.^{6,15} In cases of dry tap, for example myelofibrosis aspiration of BM is often unsuccessful because of considerable fibrotic changes and replacement of hemopoietic cell clusters onto reticulin and collagen fibers. In the study by Lozynskyy and his coworkers, BM and PVB samples from patients with myelofibrosis with cytogenetic analysis of unstimulated PVB samples culture was unsuccessful in all 10 patients due to either insufficient quantity or quality of mitotic division. When they set up cell cultures of PVB leukocytes stimulated in vitro with G-CSF all 31 patients resulted in good metaphase with successful karyotyping.¹⁸ PVB may be used for hematological malignancies keeping in mind that the abnormal clone may not be identified in such specimens as frequently as in bone marrow.¹⁴ BM samples that have been contaminated with blood during aspiration might lack an adequate number of spontaneously dividing cells. Care should be taken to avoid hemodilution of the sample. For this reason, it is important that the cytogenetics laboratory receive the first few milliliters of the bone marrow tap.^{4,19}

In our study, the incidence of UK was highest (41%) for acute lymphoblastic leukemia followed by 18% in MDS/MPN and 6% in AML. Santos et al. reported a higher UK rate in AML (13.3%) and MDS (16.4%).⁶ In their study, Medeiros and his coworkers have reported an UK incidence in AML as 10% and that it is related to poor prognosis.⁸ They have stated that UK occurs more commonly in older patients, predicts poor response to chemotherapy, and should be considered a high-risk feature. UK is seen in 6–7% of patients with MDS, mainly where marrow is fibrotic or hypocellular.²⁰ Study of FISH with MDS cases with G-banding failure done by Yang and his coworkers did not identify abnormalities with poor prognosis and none of the patients had features of high risk MDS by morphologic criteria suggesting that this finding is

associated with indolent forms of MDS.⁹ This was, in contrast, to study by Cervera and coworkers who reported that unsuccessful conventional karyotyping in MDS was associated with worse survival compared to normal karyotyping.¹⁰ In the 'Others' category, most of myeloma cases failed to grow due to low dividing capacity of plasma cells.

As per literature, a concentration of 1 million cells per ml of medium is optimal and most laboratories suspend the sample in 5–10 ml growth medium.^{6,21} Low cellularity was seen in 20% of the samples received in our lab. Out of them, 59% (119/201) showed UK as compared to 3% with high cellularity. This was found to be statistically significant. Similar results were reported by Santos and coworkers who reported association of low cellularity with higher frequency of UK.⁶ As per the guidelines if cellularity is low, a culture of lower volume should be set up in order to maintain the cellular concentration.^{21,22} At least two different cultures are recommended, using two different media or two different culture times. Although insufficient or poor-quality samples can sometimes fail to provide enough mitotic divisions, the high-count samples are most likely to fail completely. It is due to the fact that majority of these cells are incapable of division, and their presence inhibits the few remaining cells that can divide.²³ High cellularity was associated with 3% UK in our study. Out of the 3% of UK seen in samples with high cellularity, we found that highly cellular PVB had more failure rate (33%) than BM with higher cellularity (2.5%). This is similar to findings of Santos et al who reported UK in 3.9% of BM with high cellularity and 41.9% in PVB with high cellularity.⁶

CONCLUSION

Conventional cytogenetics provides support for a malignant or premalignant hematological condition and provides important prognostic and therapeutic information. It is helpful in predicting initial response to therapy, duration of remission and survival. Pre-analytical parameters impact the success of conventional cytogenetics. As per the present study, unsuccessful conventional karyotyping (14% in the present study) is directly related to pre-analytical parameters like time to process, nature of sample and cellularity. Therefore, bone marrow should be preferred for cytogenetics, samples should be transported to lab as soon as possible and correct volume of samples should be used to set up culture in order to get a better success rate. To conclude, the authors believe that collection of an adequate and good quality sample is of paramount importance on which success of karyotyping of hematological malignancies depends.

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Table 1: Pre-analytical variables and association with karyotype success

Variable	Total	Karyotype				p-Value
		Successful		Unsuccessful		
		n	(%)	n	(%)	
Time to process						
<24h	720	634	88	86	12	0.003*
>24h	300	243	81	57	19	
Type of sample						
Bone marrow	941	829	88	112	12	<0.001*
Peripheral venous blood	79	48	61	31	39	
Diagnosis						
AML	287	272	94	15	6	0.015*
ALL	156	92	59	64	41	
MDS	109	97	89	12	11	
MDS/MPN	34	28	82	6	18	
MPN	338	327	97	11	3	
OTHERS	96	61	64	35	36	
Cellularity						
>7 x 10 ³ /μl	819	795	97	24	3	<0.001*
≤7 x 10 ³ /μl	201	82	41	119	59	
TOTAL	1020	877	86	143	14	

*p-Value <0.05