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Pre-analytical Factors Affecting Cytogenetic Cell Culture Yield in Haematological Malignancies

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Abstract

Objective: To determine the association between different factors and the yield of cytogenetic culture in-terms of failure in samples drawn from patients suffering from haematological diseases.
Methodology: This descriptive cross-sectional study was conducted in Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi from Dec 2021 to Sep 2022. Two hundred and twenty six peripheral blood and bone aspirate samples received for cytogenetic culture. Samples from patients aged between 5 and 70 years, of both genders, suffering from a primary haematological disease were included. Frozen samples, or those with a quantity less than 1 mL were excluded. Patients were documented for demographic data, disease and sample characteristics. All samples were cultured and assessed for success of culture. Data was analyzed using SPSS 26.0.
Results: The sample had a median age of 34.5 (7 - 64) years, of whom 123 (54.4%) were male. Samples with culture failure had significantly lower volumes of receipt with a mean value of 2.10 ± 0.98 mL versus 3.52 ± 0.99 mL in cultures that were successful, (p<0.001). Samples that were partially clotted also had a higher incidence of cell culture failure, (p<0.001), with only 8.8% of samples showing clotting in those that were successful versus 26.9% that had cell culture failure. Additionally, cell culture had a higher chance of yielding a successful result if it was received for processing within twenty-four hours of withdrawal, with the success rate decreasing with the passage of time especially past the seventy-two-hour window, (p<0.001).
Conclusion: Delayed sample dispatch, clotted samples and inadequate volume are important factors associated with the failure of cell culture for cytogenetics.
Keywords: Cytogenetic Culture Failure, Haematological Diseases.

Introduction

The normal human diploid cell contains twenty-two pairs of autosomes and one pair of sex chromosomes which can be associated with abnormalities in both structure and number.[1] Cytogenetic analysis involves techniques such as karyotyping, fluorescence in-situ hybridization (FISH), copy number variant (CNV) microarrays and next-generation sequencing (NGS).[2] Some of these techniques are completely dependent on an adequate cultures of cells, while the newer techniques such as NGS do not strictly require cell cultures, but diagnostic yields are improved if these techniques are coupled together.[2,3] Cell cultures and their subsequent analysis are used to establish disease diagnoses, such as prenatal detection of β-thalassemia, in determining various facets of management in haematological malignancies such as classifying malignancies, detecting targets for drug therapy or determining prognosis, as well as a number of other uses.[1,4-6]

Preparing good cultures are key to obtaining accurate test results and the cell culture failure rate has been estimated at approximately 10% of all cultures performed, with guidelines recommending that a failure rate exceeding this number may be associated with serious flaws in the process of collection, transport, storage and culture of the sample.[7] Factors associated with failure of cell culture include inadequate or insufficient sample collection, clotting within sample, inappropriate buffering to control pH of the sample or culture medium, decreased number of metaphases, poor quality metaphases or banding, delays in receipt of sample, improper storage or delay in initiating culture, or inordinately low or high cell counts.[8,9] Failure of cytogenetic cultures poses the risk of dire consequences for the patient in-terms of the final diagnosis given, the management offered, as well the prognosis expected, which is particularly true for haematological and lymphoid malignancies.[5,10]

This study was conducted with the aim of determining the frequency of failed cytogenetic cell cultures in our setup

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as well as the factors associated with failure of such cell cultures. The former will help us to understand whether the practices adopted in our military setups in this matter are comparable to international guidelines, while the latter will help to identify factors that can be targeted to reduce the frequency of failed tests. This, in-turn, will enable us to correctly diagnose and manage patients with greater accuracy and efficiency. Moreover, the process of performing cell cultures is resource intensive: minimizing the incidence of cell culture failure will help save precious resources by reducing the requirement for re-testing.

Methodology

We conducted this descriptive cross-sectional study between Dec 2021 and Sep 2022 in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi on the cytogenetic culture samples of 226 patients, after obtaining consent for use of their data from them. The samples were drawn from patients who required cytogenetic culture of cells for a primary haematological disorder. Sample selection was carried out via non-probability, consecutive sampling. The WHO sample size calculator was used to calculate the sample size keeping a confidence level of (1-α) of 95%, an absolute precision (d) of 0.05 and an anticipated population proportion (P) of 0.179, which was the percentage of sample for whom the cause of culture failure was a delay in processing of samples, from Martinovic et al.[11]

Inclusion Criteria: Samples drawn from patients aged between 5 and 70 years, of both genders, who were suffering from a primary haematological disease, were included in the study.

Exclusion Criteria: Patients with suspected congenital disorders, samples which were not labeled correctly, did not have the appropriate clinical information, were completely clotted or frozen or were less than 1 mL, samples drawn from patients on chemotherapy or corticosteroids were excluded.

Patients were documented for demographic data such as age and gender. Primary diagnosis was also recorded at this point. Both peripheral blood and bone marrow aspirate samples were received in heparinised tubes containing between 1 to 5 mL of blood, transported at room temperature if the sample was drawn the same day, or refrigerated if it was drawn earlier. We considered samples which were less in quantity, contained partial clotting or were received late, samples which are usually rejected, to better understand the effect of these properties on our results. All samples were cultured for 48 hours in 10 mL of Roswell Park Memorial Institute (RPMI)-1640 cell culture media, with added fetal bovine serum in a concentration of 10%. After 48 hours, the samples were harvested, banded and analyzed. Time period to sample receipt was defined as the time taken from drawing of the specimen from the patient, to receipt in the cytogenetic lab and was stratified into hours. Cytogenetic cell culture success was defined as the acquisition of at least twenty cells in metaphase in early hybridization in a single high-power field using a light microscope.

Data was analyzed using the Statistical Package for the Social Sciences version 26.0. Mean and standard deviation was calculated for quantitative variables specifically patient age, volume of the sample, number of metaphases in the sample and cell counts on culture. Qualitative variables like gender, diagnosis, origin of the sample, partial clotting within the sample, time period to start sample receipt, whether sample was transported at the appropriate temperature, quality of the metaphases and banding were recorded in terms of frequency and percentage. Lastly, all samples were recorded for whether the cytogenetic culture was a success or not, i.e., the sample under study yielded at least twenty cells in good quality metaphase. Patients were divided into two groups: one with successful cell cultures and the other without. Quantitative variables were compared across groups using the independent samples *t*-test while the chi square test was used for qualitative variables and a *p*-value of $\leq$ 0.05 was considered significant.

Results

We conducted this study on a total of 226 cytogenetic samples, each sample being drawn from a separate patient. The study population had a median age of 34.5 (7 - 64) years, of whom 123 (54.4%) were males. A total of 55 (24.3%) patients were diagnosed as chronic myeloid leukaemia (CML), 50 (22.1%) suffered from acute lymphoblastic leukaemia (ALL), 41 (18.1%) from acute myeloid leukaemia (AML), while 34 (15.0%) had developed aplastic anaemia, 18 (8.0%) had chronic lymphocytic leukaemia (CLL), and myelodysplastic syndrome (MDS) and myelofibrosis were diagnosed in 17 (7.5%) and 11 (4.9%) cases, respectively. Table-I shows

the patient characteristics distributed according to gender.

Table I: Patient Characteristics According to Gender.		
Variable	Male (n=123)	Female (n=103)
Gender	123 (54.4%)	103 (45.6%)
Mean Age (years)	32.43 ± 16.60	35.67 ± 16.61
Diagnosis		
Chronic Myeloid Leukaemia	33 (26.8%)	22 (21.3%)
Acute Lymphoblastic Leukaemia	25 (20.3%)	25 (24.3%)
Acute Myeloid Leukaemia	16 (13.0%)	25 (24.3%)
Aplastic Anaemia	21 (17.1%)	13 (12.6%)
Chronic Lymphocytic Leukaemia	10 (8.1%)	8 (7.7%)
Myelodysplastic Syndrome	12 (9.8%)	5 (4.9%)
Myelofibrosis	6 (4.9%)	5 (4.9%)

Table II: Sample Characteristics according to Gender.		
Variable	Male (n=123)	Female (n=103)
Mean Sample Volume (mL)	3.25 ± 1.22	2.91 ± 1.12
Clotting Within Sample	17 (13.8%)	15 (14.6%)
Time Period to Sample Receipt		
< 24 Hours	53 (43.1%)	42 (40.8%)
24 – 48 Hours	35 (28.5%)	36 (35.0%)
>48 – 72 Hours	26 (21.1%)	15 (14.5%)
>72 Hours	9 (7.3%)	10 (9.7%)
Sample Origins		
Peripheral Blood	77 (62.6%)	64 (62.1%)
Bone Marrow Aspirate	46 (37.4%)	39 (37.9%)
Temperature on Transport Adequate	107 (87.0%)	90 (87.4%)
Number of Metaphases per High Power Field	28.84 ± 15.05	26.18 ± 13.58
Cell Culture Success	88 (71.5%)	71 (68.9%)

Table II displays the sample characteristics. The mean sample volume, both blood and bone marrow aspirate, was 3.10 ± 1.19 mL. A total of 32 (14.2%) samples were partially clotted. A total of 95 (42.0%) samples were received within twenty-four hours, 71 (31.4%) were received between twenty-four and forty-eight hours of drawing, while samples where between forty-eight and seventy-two hours, and over seventy-two hours had elapsed from drawing of sample accounted for 41 (18.1%) and 19 (8.4%) samples. A total of 141 (62.4%) samples received were of peripheral blood, while 85 (37.6%) were bone marrow aspirates. A total of 29 (12.8%) samples were not transported at the adequate temperature. The mean number of metaphases per high power field for the sample was 27.63 ± 14.43. Cell culture was successful in 159 (70.4%) cases.

Table-III shows the association of various factors with successful cell cultures. Lower sample volumes were associated a higher incidence of failed cultures, (p<0.001). Samples that were partially clotted also had a higher incidence of cell culture failure, (p<0.001), while a cell culture had a higher chance of yielding a successful result if it was received for processing within twenty-four hours of being drawn, with the success rate decreasing with the passage of time, (p<0.001).

Table III: Association of Factors with Cell Culture Success.			
Variable	Culture Success (n=159)	Culture Failure (n=67)	p value
Gender			
Male	88 (55.3%)	35 (52.2%)	0.688
Female	71 (44.7%)	32 (47.8%)	
Age	34.33 ± 17.26	32.91 ± 15.16	0.560
Diagnosis			
Chronic Myeloid Leukaemia	40 (25.2%)	15 (22.4%)	0.219
Acute Lymphoblastic Leukaemia	35 (22.0%)	15 (22.4%)	
Acute Myeloid Leukaemia	32 (20.1%)	9 (13.4%)	
Aplastic Anaemia	22 (13.8%)	12 (17.9%)	
Chronic Lymphocytic Leukaemia	13 (8.2%)	5 (7.5%)	
Myelodysplastic Syndrome	13 (8.2%)	4 (6.0%)	
Myelofibrosis	4 (2.5%)	7 (10.4%)	
Mean Sample Volume (mL)	3.52 ± 0.99	2.10 ± 0.98	<0.001
Clotting Within Sample	14 (8.8%)	18 (26.9%)	<0.001
Time Period to Sample Receipt			
< 24 Hours	79 (49.7%)	16 (23.9%)	<0.001
24 – 48 Hours	52 (32.7%)	19 (28.4%)	
>48 – 72 Hours	21 (13.2%)	20 (29.9%)	
>72 Hours	7 (4.4%)	12 (17.9%)	
Sample Origins			
Peripheral Blood	95 (59.7%)	46 (68.7%)	0.207
Bone Marrow Aspirate	64 (40.3%)	21 (31.3%)	
Temperature on Transport Adequate	137 (86.2%)	60 (89.6%)	0.487

Discussion

The successful culture of target cells for cytogenetic evaluation is of critical importance in the diagnosis and establishment of prognosis of many disorders, both

congenital and acquired. This testing is expensive, especially in resource-poor countries, and re-culturing of samples in-case of cell culture failure represents a potentially avoidable drain on the financial resources of an already strained healthcare system in the developing world. Thus, determining the factors responsible for cell culture failure is paramount prior to initiating measures to mitigate their affects and prevent the wastage of precious resources.

Our study demonstrated that advancing age did not have any effect on the success rate of cell culture, (p=0.560). Alzer et al reported that there was a decreased chance of acquiring an adequate culture of somatic cells for analysis as the age of the donor advanced, which was at odds with our study,[12] however, Phipps et al reported, in their review, that while there was some data to suggest that advancing age was associated with cell culture failure, but this was not consistently demonstrated across literature.[13] We believe that this variability in results is based on two factors:1) the type of cell being cultured and 2) the definition of a successful culture, with some studies having stringent criteria for culture failure such as loss of certain membrane proteins despite the acquisition of viable cells with intact nuclear material.[12,13]

Our study showed that gender did not have any statistically significant effect on the success of cytogenetic cell culture, (p=0.688). While an exhaustive literature search did not reveal a study which reviewed the effect of gender in this aspect, Fosset et al looked at the effect of gender on cell culture of mesenchymal stem cells and found that gender did not have an effect on the degree of cell proliferation within a human cell culture, which was consistent with our study.[14]

Sample origin did not appear to have an effect on the success of cell culture in our study, (p=0.207). This was at odds with Martinovic et al, who noted that bone marrow aspirate was associated with a higher incidence of cell culture failure.[11] Conversely, Asadi-Fakhr et al reported that bone marrow aspirate was associated with a better frequency of yielding a successful cytogenetic cell culture with more accurate evaluation as compared to peripheral blood.[15] We believe these variations in results are attributable to the manner in which the samples are drawn, including needle gauges, patient cooperation as well as the experience of the staff that is drawing the sample and, in practice, both sources of sampling should give equivalent results.

Samples that were lower volume had a higher chance of failing to yield a successful culture, (p<0.001), which was in accordance with Martinovic et al.[11] Howe et al recommends that a certain minimum number of cells are required for the cell culture and subsequent nucleic acid to succeed, which is not possible if the sample is of a low volume.[16,17] This inadequacy of volume also applies to clotting within the sample, which captures viable cells within the clot which become unavailable for culture.[18] The presence of clotting within a sample was associated with a higher frequency of yielding a failed culture, (p<0.001), which is in accordance with existing studies.[11] In fact, St-Antoine et al has suggested that the use of fibrinolytics to dissolve clots and release viable cells for subsequent cell culture and cytogenetic analysis is a viable strategy in utilizing clotted samples.[18]

Cell culture had a higher chance of success if it was received for processing within the first twenty-four hours of being drawn from the patient, with the culture having a decreasing chance of success as more time passed, (p<0.001), which was in agreement with existing studies on the matter,[11] however, Santos et al noted that there was no difference with regards to success of cytogenetic cell culture between samples received within 24 hours and those that were drawn over 72 hours ago.[19] We believe this difference has arisen, in part, due to the characteristics of the primary disease for which cytogenetic studies were being carried out: Santos et al reviewed culture success in patients with acute myeloid leukaemia while our study population had a great deal of heterogeneity with regards to diagnosis, and it is proposed that myeloblastic cells tend to be more hardy to passage of time.[19]

Lastly, our cell culture failure rate was 29.6%, while the recommended failure rate for a cytogenetics laboratory should not be more than 10%.[11] This can clearly be attributed to our study parameters wherein we chose to accept samples with low volumes, those that were delayed and even those with partial clotting, to better understand the role of these factors in cell culture success.

STUDY LIMITATIONS: This was a single-center study, which was also limited by its relatively small sample size. Moreover, it is unclear whether the anticoagulant present within the sample bottle has any effect on the technique of cell culturing, which

requires further study. Additionally, we limited our study to cytogenetic culture from patients afflicted by acquired disease: cytogenetic cultures failures may be associated with other factors in patients with congenital disease. Lastly, we performed these test on samples drawn from either peripheral blood or the bone marrow; cytogenetic cell cultures drawn from other tissue such as solid tumors may not necessarily be affected by the same factors.

Conclusion

Cytogenetic studies form an integral part of the diagnosis and management of a number of diseases. Effective use of this diagnostic modality requires acquiring an adequate cell culture with the requisite metaphases which, in turn, is directly linked to the volume of sample available for processing, the quality of the sample particularly the presence of clots, as well as the time taken for the sample to reach for processing. Suitable education needs to be provided to sample collection departments to facilitate in the mitigation of the negative effects of these factors, to limit the frequency of unsuccessful cell cultures.

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