



CULTURE FAILURE AND SUBOPTIMAL ANALYSIS IN CYTOGENETICS: A DATA REVIEW AS A QUALITY IMPROVEMENT METRIC FROM A RESOURCE-LIMITED COUNTRY

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Abstract

Background: Conventional cytogenetic failures can affect hematological cancer diagnosis and prognosis. Resource-constrained regions without contemporary genetic testing facilities may experience this impact more. Cultural failure (CF) and suboptimal analysis (SOA) data can identify problems and set standards. Thus, a cohort was created to find areas for improvement and reduce negative contributing factors, making this cytogenetic technique more accessible and cost-effective. A retrospective study at the Cytogenetics lab of Indus Hospital, Karachi, analyzed 1234 blood and bone marrow samples from Jan 2021 to Mar 2023. CF meant no growth, while <20 metaphases were labeled suboptimal. IBM SPSS 24.0 was used for analysis, employing chi-square to confirm factor-karyotyping associations, with $p < 0.05$ indicating significance.

Results: Of 1234 samples, 1110 (90%) were bone marrow and 124 (10%) were peripheral blood. There were 32/1234 (2.6%) CF cases; all found in bone marrow samples, making the true incidence 32/1110 (2.9%). No CF observed in peripheral blood samples tested for constitutional disorders. Additionally, chromosomal analysis quality was assessed. SOA occurred in 105/1234 (8.5%) instances, with 58% having poor morphology and 31% having a low mitotic index. Among 137 CF and SOA patients, 134/1110 (12%) were found in marrow and 3/124 (2.4%) in blood.

Conclusion: Lower CF rates suggest that appropriate standards and implementation of quality management protocols can reduce cytogenetic failure rates despite newly established services in a resource-constrained setting.

Keywords: Cytogenetics, Culture failure, Suboptimal, Pre-analytical

Background

Conventional metaphase karyotyping is a frequently used technique for detecting chromosomal abnormalities such as aneuploidy, translocations, and deletions. It helps with the diagnosis, prognosis, and improvement of treatment options for a wide range of benign and malignant disorders [1]. As

stated by the WHO classification of hemato-lymphoid cancers, it is one of the primary methods for categorizing hematological malignancies [2]. In diseases such as leukemia and lymphoma, both numerical and structural chromosomal aberrations influence prognosis and therapy decisions [3, 4]. Karyotypes can be obtained from a variety of tissues, with bone marrow aspirate preferred in hematological cancers and peripheral blood favored in congenital disorders. Prenatal diagnosis, on the other hand, necessitates the use of amniotic fluid or chorionic villous materials [5-7]. The generation of a karyotype requires the setting up of a short-term culture of cells and their arrest in the metaphase stage of the cycle. This is followed by a series of processes, including nucleus extraction, fixation, and the preparation of Giemsa-stained slides for bright-field microscopy examination, or captured image analysis [7, 8]. The effectiveness of this approach is contingent on cultural success, availability of metaphases, and morphological adequacy. The results can be influenced by the underlying diagnosis, inherent biological features of the leukemic clone, sample conditions, and transit time [9, 10]. Furthermore, the culture medium and techniques used in chromosomal banding might have an impact on the outcome [9]. According to standards, the failure rate for bone marrow and neoplastic blood samples should not exceed 10% [11]. In previously published studies, the reported success rates for karyotyping varied from 78 to 90% depending on the underlying disease and sample conditions [10, 12].

Failures in conventional cytogenetic testing can have major consequences for diagnostic and prognostic information, particularly in hematological cancers. This impact can be more drastic in resource-constrained laboratories like ours, where modern complementary genetic testing facilities are unavailable. As a result, vigilance is essential to reduce the rate of failed cytogenetics. In addition to other quality measures, culture failure (CF) and suboptimal analysis (SOA) data can be utilized as quality improvement indicators. The analysis and information gathered from this data can aid in determining the underlying causes of failure, developing processes to overcome those causes, and establishing a benchmark. In light of this, a cohort was planned to examine the prevalence and associated causes of failure and suboptimal analysis in our circumstances. This research will assist us in identifying areas for improvement and developing a strategy to minimize the negative contributing elements in order to maximize the success, accessibility, and cost-effectiveness of this cytogenetic approach.

Methods

Study Design:

This is a retrospective observational study conducted at the Cytogenetics laboratory of Indus Hospital and Health Network (IHHN), Karachi. IHHN is one of the country's major tertiary care nonprofit nongovernmental organizations. After gaining ethical approval (IRB# IHHN_IRB_2022_09_005), the hospital's electronic medical record (EMR) was used for data collection. A total of 1234 patients were evaluated for conventional cytogenetics. This includes bone marrow samples collected between January 2021 and March 2023, as well as blood samples from March 2022 to March 2023. The karyotypes of blood samples were standardized and made available in March 2022. Acceptance criteria for sample transit time, quantity, and count were relaxed due to clinical constraints, such as the need to repeat invasive procedures and the urgency to treat patients in unstable condition. Therefore, all samples were processed irrespective of any violation in pre-analytical variables except hemolyzed and clotted samples which were rejected.

Cell culture:

All samples were processed in Biosafety cabinet using sterile techniques. Two cultures were processed for each specimen using 10 ml of complete media composed of Roswell Park Memorial Institute (RPMI) media supplemented with fetal bovine serum (FBS) without phytohaemagglutinin (PHA) and with PHA for constitutional disorders, L-glutamine, and antibiotic (Penicillin/Streptomycin). Quality control of media was ensured by parallel testing with old lot to see if it supports cell growth and 5ml media was sent to Microbiology lab for bacterial culture to verify

sterility of the reagent. Metaphases were arrested by colcemid after culture and then treated with a hypotonic solution, Potassium chloride (KCL). Fixation with Carnoy's fixatives (3-parts methanol and 1-part glacial acetic acid) was then performed. After fixation, slides were made by manual dropping and GTG banding was done. To verify optimal quality of banding, Vancouver method or band 10 method was used. Minimum band level (Band resolution) of 400 is required for bone marrow while 550 for peripheral blood. The Cytovision MB8 program, a semi-automated capturing system was used to capture images of metaphase spreads from all prepared slides. Two reviewers analyzed a minimum of 20 cells for metaphase spread. We categorized the samples as CF if they showed no evaluable metaphases. An analysis was considered suboptimal for cases showing less than 20 metaphases for examination or having poor morphology (PM) of the chromosome. PM chromosomes were excessively clustered, lacking dispersion, and lacked a distinct banding pattern. The findings were documented using the International System for Human Cytogenetic Nomenclature (ISCN-2020).

Analytical variables:

The following analytical factors were investigated: 1) time from collection to sample processing (more or less than 48 hours); 2) sample quantity (< 1 ml or ≥ 1 ml); 3) sample cellularity/white cell count ($< 4 \times 10^9/L$ or $\geq 4 \times 10^9/L$); 4) specimen type (BM or PB); 5) sample received from an in-house or out-reach department, and 5) diagnosis of patient.

Data Analysis:

Data were entered and analyzed using SPSS, version 24.0 (IBM Corp., Armonk, NY). Frequency and percentage were evaluated for different analytical variables such as sample type, transit time, etc. The distribution of cytogenetic results and possible reasons for failure were also computed. The chi-squared test/Fisher's exact test or likelihood ratio test was used to verify associations between variables and karyotyping. A p-value of < 0.05 was considered significant.

Results

A total of 1234 cases were included in this study for the assessment of cytogenetic results. Of the 1234 samples collected, 1110 (90%) were bone marrow and 124 (10%) were peripheral blood (Figure 1).

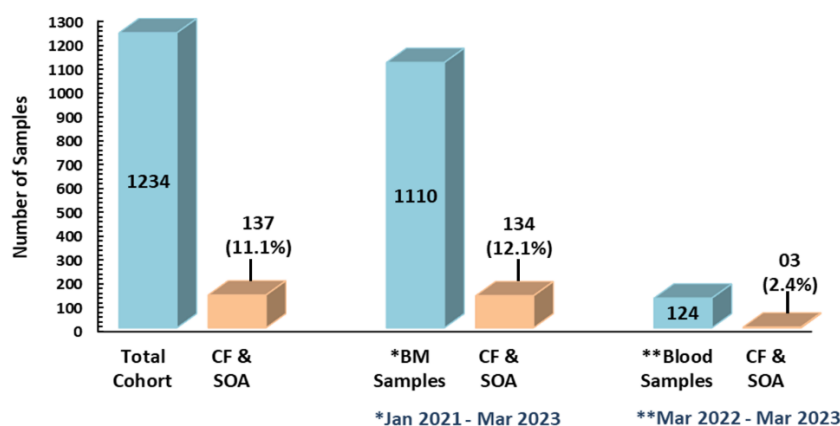


Figure 1. Distribution of cytogenetic data according to sample type
CF: Culture failure, SOA: Suboptimal analysis.

There were no evaluable metaphases (culture failure – CF) in 32/1234 (2.6%) cases and all of those detected in bone marrow samples, resulting in a true incidence of 32/1110 (2.9%). Constitutional blood testing revealed no CF in 124 samples. Furthermore, an analysis was performed to evaluate the quality of the chromosomal analysis. SOA is seen in 105 out of 1234 patients, accounting for 8.5% of the total. Among these instances, 58% exhibited PM and 31% had a low mitotic index (LMI) (Figure

2). Out of 105 instances with SOA, 102 cases were found in bone marrow, accounting for 102 out of 1110 cases (9%), while 03 cases were found in blood samples, resulting in an incidence of 03 out of 124 cases (2.5%).

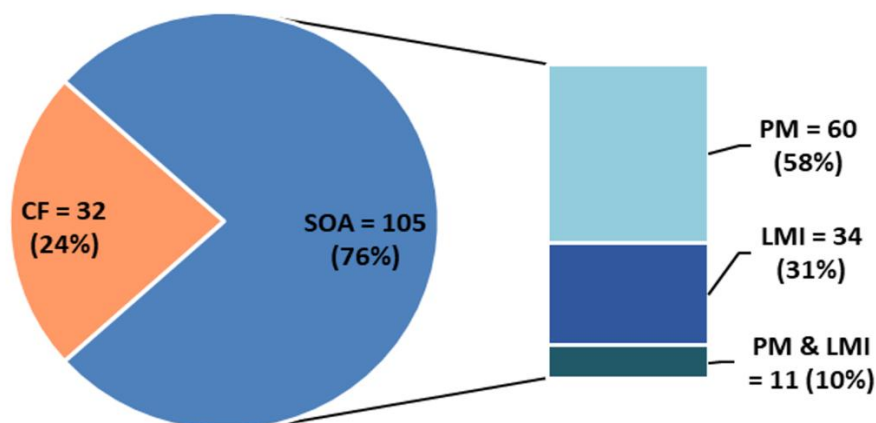


Figure 2. Distribution of CF and SOA

CF: Culture failure, SOA: Suboptimal analysis, PM: Poor morphology; LMI: Low mitotic index.

The correlation between clinical features and other pre-analytical factors with CF and SOA is presented in Table 1. There is a significant association between variables such as diagnosis (p-value: <0.0001), processing time (p-value: <0.0001), and sample location (p-value: <0.001) and the incidences of CF and SOA.

Table 1. Association of clinical and pre-analytical variables with CF and SOA.

Variable	Total (N=137)	Culture Failure (N=32)	Sub-optimal analysis (N=105)	P-value
Gender				
Male	78	16 (50%)	62 (59.8%)	0.328 ^a
Female	59	16 (50%)	43 (40.2%)	
Diagnosis				<0.0001 ^{a**}
B lymphoblastic leukemia	87	11 (34.4%)	76 (72%)	
Acute leukemia*	17	9 (28.1%)	8 (7.6%)	
Unexplained Cytopenia	17	9 (28.1%)	8 (7.6%)	
Myeloproliferative neoplasm	7	1 (3.1%)	6 (5.7%)	
Others	9	2 (6.3%)	7 (6.6%)	
White cell count[^]				0.337 ^a
Low	41	12 (40%)	29 (28.7%)	
Normal	73	16 (53.3%)	57 (56.4%)	
High	17	2 (6.7%)	15 (14.9%)	
Sample quantity				0.225 ^b
≤1ml	20	7 (21.9%)	13 (12.3%)	
>1ml	117	25 (78.1%)	92 (87.7%)	
Time to process				<0.0001 ^{a**}
<48 hours	103	17 (53.1%)	86 (82%)	
≥48 hours	34	15 (46.9%)	19 (18%)	
Sample received from				<0.001 ^{a**}
In-campus services	100	15 (46.9%)	85 (81%)	
Out-reach services	37	17 (53.1%)	20 (19%)	

*Acute leukemia with undetermined phenotype, ^WCC is not documented in six cases

a = Chi-square test, b = Fisher's exact test, ** Significant value

Discussion

Cytogenetics plays a key role in the diagnosis, prognosis, and treatment monitoring of both benign and malignant diseases. Many chromosomal abnormalities have been identified as potential indicators of prognosis and outcome in hematologic cancers. However, a variety of pre-analytical and analytical factors contribute to culture failure and suboptimal analysis, affecting its utility and reliability.

Within our cohort, a total of 32 samples (2.9% of the total) were unable to produce metaphase cells that were appropriate for assessment, and as a result, these samples were categorized as culture failures. All of these examples of CF were documented in bone marrow samples obtained for various benign and malignant hematological diseases. Testing for constitutional cytogenetics began in March 2022, with peripheral blood serving as a sample source. Since then, we have analyzed 124 blood samples for constitutional indications, but we have not reported any of them as CF. Our cohort exhibits a significantly lower incidence of CF compared to the reported literature i.e.10–20% [12-14]. The analysis addressed both general and technical factors, which could explain the low incidence of CF (Table 2). Another reason can be the homogeneity of the indication, since the majority of the samples obtained from the pediatric oncology unit with the diagnosis of B lymphoblastic leukemia.

Table 2. Possible reasons of achieving better KPIs in our cytogenetics laboratory.

General aspects	Technical aspects
Education and training 1. Regular CMEs 2. Established policies and procedures 3. Periodic training and competency assessment	Laminar flow hood 1. Use of laminar flow hood (Class-II) to reduce contamination 2. Use of UV light to sterilize the culture hood
Adherence to protocols 1. Adherence to SOPs 2. Updating protocols	Media composition and aliquoting 1. Appropriate media composition with calculated amount of different reagents and suitable antibiotics 2. Media aliquoting before storage to prevent multiple thaw/freeze cycles
Quality control 1. Established criteria for acceptable culture results 2. Regular participation in proficiency testing	Processing and Harvesting 1. Testing new lots of media and sera for sterility and ability to support cell growth 2. Recording the date the media initially utilized. 3. Harvesting samples at different times 4. Each sample should be split to be grown in separate incubators
Documentation and record keeping 1. Records for each culture including patient information, pre-analytical variables and culture conditions 2. Access to patient record for adequate clinical information 3. Continuous test volume monitoring to manage workloads and staffing issues	Incubators 1. Availability of two incubators to process multiple cultures at separate location 2. Use of stainless steel incubators for easy cleaning and avoiding corrosion 3. Daily monitoring for temperature, pH and CO₂ levels 4. Using CO₂ gas analyzer to monitor CO₂ levels in incubators
Environmental controls 1. Monitored laboratory environment for temperature, humidity and air quality. 2. Regular cleaning and disinfection of workspaces	Slide making 1. Maintaining humidity and temperature in slide making area 2. Using humidifier and dehumidifier as needed 3. Assuring appropriate slide - using wet slides and maintaining 20-30° angle
Sample considerations 1. Ensure aseptic techniques and rapid transportation of the sample at ambient temperature (Do not freeze) 2. Avoid contamination of marrow sample by peripheral blood (hemodilution)	Quality control of reagent and solutions 1. Quality control of new reagent lots and changes in establishing protocol prior using. 2. Use of appropriate QC methods such as parallel testing of the current validated reagents against the new lots.
Troubleshooting protocols 1. Develop clear guidelines for common issues encountered during cell culture 2. Encourage a culture of adaptability, continuous improvement and collaboration among staff	

In cytogenetic studies, standards recommend examining at least 20 metaphases to eliminate 14% of any aberrant clone with 95% certainty [15]. However, the quality of chromosomal morphology and resolution of neoplastic metaphases is generally poor, particularly in leukemia, and repeat sampling is seldom achievable due to the early initiation of therapy. As a result, guidelines provide no suggestions about minimum banding quality. Because neoplastic samples, including acute leukemia, were the most prevalent reason for karyotype in our cohort, we used SOA as a quality measure. Among the 105 (8.5%) cases with SOA, 58% had PM, 31% had LMI, and 11% had both findings. Analysis of chromosomes with poor morphology and resolution is challenging and can cause errors in interpretation. Bone marrow aspirates are known to produce insufficient or poor-quality chromosomes. Extended exposure or high colcemid concentrations can cause condensed chromosomes, whereas low concentrations can cause extended overlapping metaphases [16, 17].

Several studies have linked CF and suboptimal chromosomal analysis to a variety of pre-analytical and analytical factors [17-19]. The current study found that B-lymphoblastic leukemia patients had the highest rates of CF and SOA, followed by other acute leukemias and unexplained cytopenias. These results are consistent with the existing literature. Unlike adult oncology services, our hospital has a well-established and active pediatric oncology section. Therefore, the majority of bone marrow samples tested for karyotype exhibited the diagnosis of acute lymphoblastic leukemia. Our hospital is a health network that collaborates with multiple out-reach centers to provide specialized diagnostic and therapeutic services. As a result, we evaluated the data for in-house versus outreach campus samples, as well as the processing time within and over 48 hours of sample collection. We received approximately 25% of the CF and SOA cases from outreach centers and processed them after 48 hours of collection in the current cohort, observing a statistically significant variation across these groups. To minimize aging of samples, the laboratory should receive specimens as soon as possible which can also prevent exposure to extreme temperatures. Similarly, using transport medium is highly recommended to reduce sample drying and maintain cell viability [18, 19].

Our study observed a significantly low incidence of CF, despite the majority of karyotype indications coming from oncology patients and processed on bone marrow aspirate samples. The probable reason for these favorable outcomes can be attributed to the stringent adherence to both general and technical testing procedures, as mentioned in Table 2. However, we need to address the challenge of transporting outreach samples and processing them promptly.

While our study provides valuable insights into our laboratory's optimal practices, we must acknowledge certain limitations. The majority of the patients in our study were pediatric B-ALL patients, which limits the applicability of our low CF rate to other hematological malignancies or age groups. Furthermore, the fact that we conducted our investigation at a single site may limit its representativeness to other cytogenetic laboratories. As a result, sharing our established methods and policies with other laboratories may help to increase cytogenetic testing efficiency.

Conclusion

Our study shows lower CF than reported in the literature, despite the challenges posed by limited resources and the recent establishment of cytogenetic services. These results indicate that adhering to rigorous criteria during the pre-analytical and analytical stages of sample processing, as well as ensuring effective quality controls, can significantly decrease cytogenetic failures. By implementing these measures, laboratories can improve the accuracy and reliability of their cytogenetic testing results.

Abbreviations:

CF: Culture Failure

FBS: Fetal Bovine Serum

ISCN: International System for Human Cytogenetic Nomenclature

KCL: Potassium Chloride

LMI: Low Mitotic Index

PHA: Phytohaemagglutinin

PM: Poor Morphology

RPMI: Roswell Park Memorial Institute

SOA: Suboptimal analysis

Declarations

Ethics approval: The study is approved by the Indus Hospital's ethics committee (IHHN_IRB_2022_09_005).

Availability of data and materials: The data will be made available upon request to the corresponding author.

Competing interests: None to declare.

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Authors' contributions: N.M is the principal investigator, designed the work, N.T assisted in data analysis, and prepared the manuscript with N.M, A.Z and B.A participated in data curation and analysis, S.A and B.K critically reviewed the manuscript. All the authors approved the manuscript for submission.

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