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Optimal Tissue Size for Successful Cytogenetic Yield in Lymphoma Work-Up

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Abstract The work-up of suspected lymphoma involves conventional cytogenetic analysis for diagnostic and prognostic purposes. Definitive guidelines specifying the size or quantity of tissue needed to yield successful cytogenetic results are lacking, and significant variation in the success rate is routinely observed. Our study aims to describe one medical center's experience with cytogenetic analysis of specimens submitted for lymphoma work-up and assess the optimal quantity of tissue required to yield successful cytogenetic analysis. 417 cases of suspected lymphoma over a two-year period that were submitted for cytogenetic analysis at Long Island Jewish Medical Center and North Shore Hospital were reviewed. Proportions of lymphoma and non-lymphoma cases as well as failed and successful analyses were evaluated. Specimen size measurements were reviewed to characterize how tissue size impacts cytogenetic analysis yield. 209 lymphoma and 208 non-lymphoma cases were identified. 52% of lymphoma cases and 58% of non-lymphoma cases had failed cytogenetic analysis. Size cutoff value to produce a successful cytogenetic analysis was determined to be 0.2 cm^3 (p=0.022). More than half of specimens submitted for lymphoma work-up yielded a failed study. We found that specimens submitted for analysis that were at least 0.2 cm^3 produced successful results. We hope to make providers aware of the importance of submitting adequate biopsy material to ensure yield and avoid repeat biopsy.

Keywords: *lymphoma, biopsy, cytogenetic analysis, tissue adequacy*

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1. Introduction

Cytogenetic studies have a critical role in the diagnosis and prognostication of lymphomas [1,2,3]. However, with fine needle aspiration and core biopsies becoming more common there is a potential for suboptimal specimens that yield karyotype failures. The Association of Genetic Technologists Cytogenetics Laboratory Manual states that tumor specimen size between 0.5-1.0 grams or 0.5-1.0 cm^3 (viable tissue) is sufficient in most cases, however, internal institutional policies usually state that all specimens should be accepted, regardless of size or quality, to attempt cytogenetic analysis [4]. To assess the proportion of biopsies achieving a successful karyotype result, we reviewed two years of cytogenetic information for rule-out lymphoma specimens at our institution.

2. Materials and Methods

We identified all cases of suspected lymphoma for which karyotyping was performed over two years (from January 2012 to January 2014) at our health system cytogenetic laboratory. Specimens included nodal and extranodal tissues. Final hematopathology reports were reviewed and classified as either lymphoma or non-lymphoma. Lymphoma cases were further sub-classified based on the type of lymphoma. Cases were reviewed to characterize cytogenetic analysis as successful or failed. Fisher Exact Test (2-sided) was used to examine size in relation to the success of cytogenetic analysis. This project was approved by the Northwell Health Institutional Review Board.

3. Conventional Cytogenetic Analysis

Lymph nodes submitted for cytogenetic analyses were minced manually with a scalpel or using a tissue grinder system (no enzymatic digestion). One to two milliliters of MarrowMax™ was added to create a cellular suspension. Suspension was used to initiate overnight culture and 72-hour culture with stimulation (PHA/IL2). Cultures were harvested using 0.075 M potassium chloride: 0.4% sodium citrate hypotonic agent and 3:1 methanol-acetic acid fixative. G-banded metaphases were analyzed and described using the International System for Human Cytogenetic Nomenclature [5].

4. Results

417 specimens submitted for rule out lymphoma were identified. 209 were diagnosed as lymphoma and 208 were negative for lymphoma. In total, more than half of specimens submitted for lymphoma work-up (220/401; 55%) were failures. The breakdown of cases is shown in Table 1. Of the non-lymphoma cases, cases positive for metastatic disease or extranodal tissues with non-hematopoietic pathologies (non-hematopoietic tumors including salivary gland tumors, lung carcinoid tumors, rhabdomyosarcoma, etc.; n=16) were eliminated. The 192 remaining non-lymphoma cases including benign/reactive and atypical cases, had a failure rate of 58% (112/192). All atypical cases had successful analysis. Data for the size of tissue submitted for cytogenetic analysis was available for 304 cases.

Table 1. Cytogenetic analysis failure and success rates among lymphoma and non-lymphoma cases

	Lymphoma	Non-lymphoma	Total
Cytogenetic Failure	108	112	220
Cytogenetic success	101	80	181
Total	209	192	401

Of the 209 cases positive for lymphoma, 52% (108/209) were failures and 48% (101/209) were successful. Lymphoma subtypes and number of cases are shown in Table 2, and reflect the relative frequencies of lymphoma subtypes as described in the literature: diffuse large B-cell lymphoma (29%), follicular lymphoma (15%), mantle cell lymphoma (4%), marginal zone lymphoma (9%), small lymphocytic lymphoma/chronic lymphocytic leukemia (6%), NK/T-cell lymphoma and T-cell lymphoma (5%), Hodgkin lymphoma (including nodular lymphocyte-predominant Hodgkin lymphoma and classical Hodgkin lymphoma) (16%), malignant B-cell lymphoma not otherwise specified (including B-lymphoblastic leukemia/lymphoma) (10%), composite lymphomas (4%) and others (including grey zone lymphoma and primary cutaneous follicle center lymphoma) (1%) [3]. A statistically significant size cut-off was established: successful outcomes were more likely when size exceeded 0.2 cm^3 (p=0.022), and more likely to yield a failed result when smaller (Table 3).

Table 2. Lymphoma subtypes and karyotype outcomes.

Type of lymphoma	Successful Cytogenetic analysis [n (%)]	Failed Cytogenetic analysis [n (%)]
		Normal
Diffuse large b-cell lymphoma	32/61 (52)	7
Follicular lymphoma	21/32 (66)	3
Mantle cell lymphoma	2/8 (25)	0
Extranodal marginal zone lymphoma	1/9 (11)	0
Splenic marginal zone lymphoma	1/2 (50)	0
Nodal marginal zone lymphoma	6/8 (75)	1
Small lymphocytic lymphoma/chronic lymphocytic leukemia	10/13 (77)	2
Peripheral T cell lymphoma & NK/T-cell lymphoma	4/10 (40)	0
Nodular lymphocyte-predominant Hodgkin lymphoma	3/3 (100)	2
Classical Hodgkin lymphoma	5/30 (17)	4

Table 3. Fisher Exact Test (2-sided) to analyze size of specimen submitted

	Size Submitted		No. of cases	p value
	<=0.2 cm^3	>0.2 cm^3		
Failure	58	76	134	0.022
Success	51	119	170	

5. Discussion

Definitive technical standards and general guidelines for cytogenetic analysis for most solid tumors are lacking. This was addressed by the European Journal of Human Genetics in which results of a best practice meeting to produce professional guidelines in Europe were reported. They note that success rates of cytogenetic analysis depend upon the technique and the quality and quantity of material received. Additionally, the authors note that "fresh tumor samples received in cytogenetic laboratories can be very variable in amount and quality". The ideal tumor specimen size should be 0.5-1.0 cm^3 according to the AGT Cytogenetics Laboratory Manual [4]. However, due to the increased use of fine-needle aspirations for tumor sampling, cytogenetic laboratories have had difficulty in making successful studies4. Many laboratories cite no minimum acceptable criteria about specimen size. Small specimens require prolonged culture periods to obtain adequate mitotic cells for analysis. When the sample size is very limited, for example, fine needle aspirate biopsy core needle biopsy, coverslip cultures are often successful [5,6,7].

Our proportion of failed karyotypes (220 of 401 cases; 55%) was significantly higher than expected. It is well known that karyotype failure (less than 5 non-informative metaphases or no metaphases available for analysis) occurs more frequently in fresh tissues due to non-availability of readily dividing cells; however, the failure rate in lymphoma cases in particular (52% of cases) was significantly higher than what has been reported in the literature and what we have experienced with other specimen types. For example, the failure rate for bone marrow specimens was approximately 3% at our institution. We believe the primary reason for this is suboptimal sample submission, possibly due to increased reliance on core or fine needle aspirates biopsy rather than incisional biopsies. Specimen quality data (i.e. necrosis) was not available in enough samples, and we recognize this as an important factor in whether a cytogenetic study fails or succeeds. The impact of viability therefore should be the subject of further investigation.

6. Conclusion

As a result of our findings, we established a size cutoff of 0.2 cm3 (p=0.022) which was statistically significant and associated with more successful results, as a minimum amount of tissue required to obtain successful cytogenetic results. This guideline may serve as an educational resource for clinicians and medical cytogenetics to help them provide quality medical genetic services. Additionally, we plan to use coverslip culture techniques

for smaller specimens, and success rates will be investigated after interventions are implemented. We propose this information be considered in the development of recommended guidelines.

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Statement of Competing Interests

The authors have no competing interests.

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