Challenges faced by laboratories in differentiating between non-neoplastic, reactive lymphocytes and abnormal lymphocytes, neoplastic in a blood smear

Anna Johnston[1,2]; Andrew McFarlane[1,2]; Gini Bourner[3]; Tracy Martin[4]; Ruth Padmore[5]

[1]Quality Management Program—Laboratory Services (QMP–LS), Toronto, ON,Canada; [2]Institute for Quality Management in Healthcare (IQMH), Toronto, ON, Canada; [3]Gamma-Dynacare Medical Laboratories, Brampton, ON, Canada; [4]Health Sciences North/Horizon Santé-Nord Ramsey Lake Health Centre, Sudbury, ON, Canada; [5]Ottawa Hospital-General Campus, Ottawa, ON, Canada

Introduction

Manual blood film reviews are performed after detecting abnormal counts, instrument flags or when the complete blood count (CBC) results fall outside of defined criteria.

Automated analyzers are becoming more sophisticated adding new technology and improving laboratory workflow, however, hematological abnormalities such as the presence of abnormal leukocytes, abnormal red cell and platelet morphology is still dependent on the ability of a skilled technologist to identify abnormalities and subsequently refer to a hematologist, pathologist and/or laboratory physician.[1,2]

Differentiating between non-neoplastic, reactive lymphocytes and abnormal lymphocytes, neoplastic is dependent on the an individual's experience as well as the available clinical information.[3]

Laboratories should have protocols for when a manual smear review is required, which should be based on clinical evidence or published criteria.[1] The International Society for Laboratory Hematology (www.islh.org) has published consensus guidelines recommending slide review for a first time absolute lymphocytosis (adults >5.0 × 10^9/L, >7.0 × 10^9/L in children <12 years old), and atypical/variant lymphocyte or blast flagging. The ability to differentiate between reactive (non-neoplastic) lymphocytes and neoplastic lymphocytes, during slide review can aid in a rapid diagnosis, and may be crucial for initiating prompt therapeutic interventions.

The Quality Management Program—Laboratory Services (QMP–LS) provides proficiency testing for peripheral blood morphology. A recent survey (November 2013) demonstrated some laboratories experienced difficulties distinguishing reactive lymphocytes from neoplastic lymphocytes. This poster illustrates a summary of the data obtained from the survey.

Methods

QMP–LS distributed a peripheral blood smear obtained from a patient sample diagnosed with infectious mononucleosis to assess laboratory performance on white blood cell (WBC) differential and descriptive morphology. Laboratories were provided with a typical clinical history of a young adult presenting with fever and sore throat. The laboratory data including the leukocyte count of 10.9 × 10^9/L was provided (Table 1). The monospot result (positive) was not provided.

Results

A total of 162/173 (94%) of participating laboratories included reactive lymphocytes in their WBC differential count. Of these, 151 (87.2%) laboratories included only reactive lymphocytes in their differential, and were assessed as having provided a correct differential (Figure 1).

A total of 18 (10.5%) laboratories reported the presence of other abnormal WBC and were assessed as having provided an incorrect differential. Two (1.2%) laboratories reported reactive lymphocytes and blasts, and one (0.6%) laboratory reported both blasts and neoplastic lymphocytes. Five (2.9%) laboratories reported neoplastic lymphocytes, 9 (5.2%) reported both reactive lymphocytes and neoplastic lymphocytes, and 1 (0.6%) reported neither reactive lymphocytes nor neoplastic lymphocytes.

Additionally, four (2.3%) laboratories commented that reactive lymphocytes were present, but did not include them as a separate category in the differential count.

Out of 173 laboratories, 157/163 (91%) reported a diagnosis (Figure 2). Reporting the diagnosis from a peripheral blood film is voluntary and considered an educational component of the survey; laboratories are not assessed.

Assessment of Laboratories

Following the survey, responses are analyzed and participants are assessed based on assigned values that are determined by expert laboratory value through use of confirmatory testing and/or medical diagnosis of the testing-material donor and consensus value from participants.

In this survey, 18 (10.4%) laboratories were assessed as having provided incorrect responses for various reasons (Figure 3). These laboratories were required to submit a discordant findings investigation to identify the contributing causes and to perform root-cause analysis (Table 2).

Ten (5.7%) laboratories reported misidentification of blood cell morphology as a contributing cause in their discordant findings investigation. Comments reported by laboratories included:

- Limited exposure to abnormal morphologies affects the ability of staff to remain experienced at morphological description and identification. Additional training and greater exposure to abnormal morphologies would greatly benefit staff.
- The blast cells in question were not brought to the pathologist's attention for review prior to submitting the report.
- Although the lymphocytes were recognized as abnormal, the low frequency of exposure to these types of abnormal blood films may have made it difficult to distinguish the exact abnormality that was present.

In routine practice, 166 (96%) of laboratories would have a hematologist, pathologist and/or laboratory physician review the blood film, and 163 (94%) would issue a written or verbal preliminary report to the ordering physician (Table 3).

Table 1. Laboratory Data

	Lab Data	Reference
Leukocyte count	10.9 × 10^9/L	3.5–10.5
Erythrocyte count	4.90 × 10^{12}/L	4.50–5.20
Hemoglobin	143 g/L	130–160
Hematocrit	0.42 L/L	0.370–0.490
MCV	85.7 fL	80–100
MCH	29.2 pg	25.0–35.0
MCHC	341 g/L	315–355
Thrombocyte count	40 × 10^9/L	138–380

Table 2. Summary of Discordant Findings Investigations – Contributing Causes

No. of Labs	Description
10	Misidentification of blood cell morphology
4	Over-reporting of morphology descriptive features
1	Results not correctly transcribed to analysis worksheet
1	Failed to remove the cells from Abnormal Lymphocytes and include with reactive lymphocytes before reporting
2	Other

Table 3. Participants' Responses to Questions on Laboratory Practice with Respect to Review, Referral and Reporting

In routine practice, would a hematologist, pathologist and/or lab physician (in or outside the laboratory) review this blood film?

	No. of Labs	% of Labs
Yes	166	96
No	7	4

If this blood film were referred for review, would a written or verbal preliminary report be issued to the ordering physician?

	No. of Labs	% of Labs
Yes	163	94
No	5	3
No response	5	3

Figure 1. Reporting of reactive vs. neoplastic vs. blasts

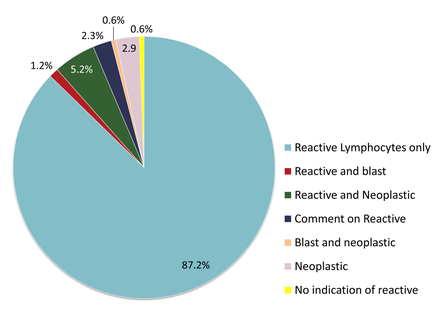


Figure 2. Reference diagnostic statements

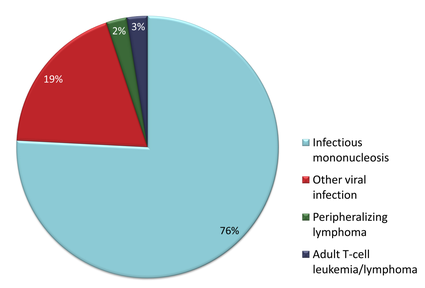


Figure 3. Laboratories submitting discordant findings investigations

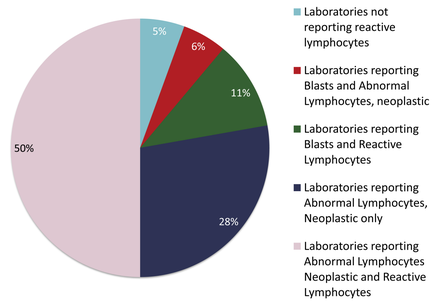


Figure 4. Marked variation in the lymphocyte morphology, including large reactive lymphocytes and small lymphocytes. (Wright-Giemsa, original magnification 100× oil immersion).

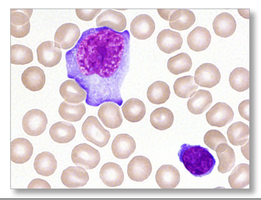


Figure 5. Reactive lymphocytes (10–25 µm in diameter) are characterized by their wide range of morphological appearance within the same peripheral blood film. These cells are reacting to an abnormal stimulus and are frequently increased in viral illness such as Epstein-Barr virus infection (infectious mononucleosis).

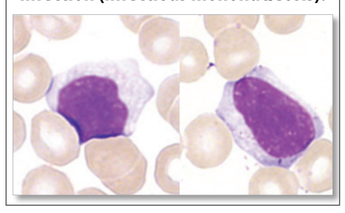


Figure 6. Rare reactive lymphocyte with clover-leaf like morphology, not to be mistaken for a "flower cell" of adult. T-cell leukemia/lymphoma. (Wright-Giemsa, original magnification 100× oil immersion)

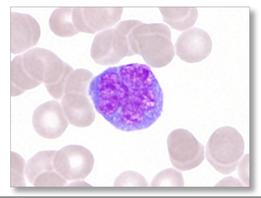


Figure 7. Rare reactive lymphocyte with more prominent nucleoli, not to be mistaken for a blast cell or a lymphoma cell. (Wright-Giemsa, original magnification 100× oil immersion).

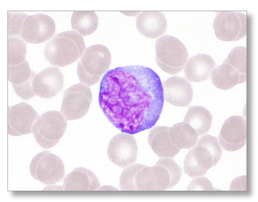


Figure 8. Reactive lymphocytes with plasmacytoid morphology

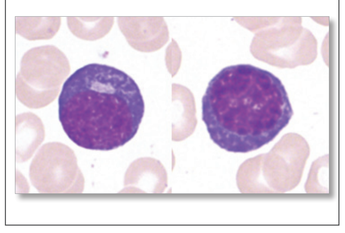
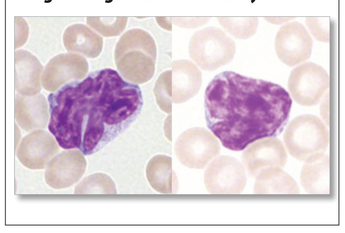


Figure 9. Abnormal lymphocyte, neoplastic are 8–30 µm in diameter and exhibit a wide variety of appearances. Any individual case tends to show a monotonous population of abnormal cells. There are usually irregular nuclei, nucleoli may be present, the nuclear:cytoplasmic ratio tends to be high and granules are rarely found.



Conclusions

The results of this proficiency testing survey highlight challenges for the morphologic identification of reactive lymphocytes (Figure 4–8) versus neoplastic lymphocytes (Figure 9) and reveals variation in laboratories' reporting practice.

Counting reactive lymphocytes separately in the differential is a simple tool that can be implemented by laboratories to alert clinicians of their presence and aid in a diagnosis. In most cases, the clinical context and morphologic appearance should enable the experienced reviewer to reliably differentiate reactive lymphocytes from neoplastic, but it is appreciated that in the real working world this may on occasion be difficult.[4] In these situations, ancillary testing such as monospot and/or flow cytometry immunophenotyping may be helpful.

The review of a blood film can be an important tool for making the correct clinical diagnosis.[5] Laboratories should have a process to ensure abnormalities found during review of a blood film get interpreted by appropriate laboratory professionals and are reported back to health-care providers.[1,5,6]

Further education in lymphocyte morphology and standardization of reactive lymphocyte reporting practice may be useful tools for laboratories to implement.

References

1. Bain, BJ. Diagnosis from the blood smear. N Engl J Med. 2005 Aug 4;353(5):498-507.
2. International Society for Laboratory Hematology, Consensus Guidelines, Suggested Criteria for Action Following Automated CBC and WBC Differential Analysis. Available from: http://www.islh.org/web/consensus_rules.php (accessed February 6, 2014).
3. van der Meer W, van Gelder W, de Keijzer R, Willems H. The divergent morphological classification of variant lymphocytes in blood smears. J Clin Pathol, 2007; 60; 838–9.
4. Hernandez JS, Dale JC, Bennet KE, Varkey P. Challenges and opportunities for medical directors in pathology and laboratory medicine standardization, integration, and innovation. Am J Clin Pathol 2010; 133:8–13.
5. Barth D. Approach to peripheral blood film assessment for pathologists. Semin Diagn Pathol. 2012 Feb;29(1):31–48.
6. Gulati G, Song J, Florea AD, Gong J. Purpose and criteria for blood smear scan, blood smear examination, and blood smear review. Ann Lab Med. 2013 Jan;33(1):1–7.