Examining Our Laboratory Results Through Participation in Multinational Material Exchange Studies

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Original Research Article

ABSTRACT

Aim: The aim of this study is to determine the quality of our tissue processing through participation a multicentre research programme as part of external quality control.
Study Design: A analytical retrospective study.
Place and Duration of Study: Department of Pathology University of Calabar Teaching Hospital, July 2019.
Methodology: An analytical study reviewing the performance of lymphoma tissue contributed to a Sub-Saharan African Lymphoma consortium study commissioned in 2008/2009 for which results were published in 2012. Twelve formalin fixed paraffin embedded lymphoma tissue were tested with a panel of 40 immunohistochemistry antibodies. The tissues were cut into 480 cores placed on slides before the test.
Results: The tissues were from 5 women and 7 men. The mean age was 37years, median age 45 years and modal age was 60 years. Twenty six percent of the sectioned cores lifted at test and could not therefore produce results. The reason for the lift off was tissue brittleness. Seventy four percent (74%) had intact cores on slides and produced a staining reaction although fragile antibodies like Ki 67 and bcl6 produced non reliable results while hardy antibodies like CD20 were more reliable.

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Conclusion: The quality of histopathology biopsy results in the Department of Pathology University of Calabar teaching hospital needs to be improved. The strategies to achieve this involves the institution of continuous quality control and quality assurance.

1. INTRODUCTION

Approaches to instituting quality control (QC) and quality assurance (QA) in Anatomic pathology may vary, but the result is the same. A laboratory for instance set up its QC committee with a mandate to assess two levels of QC, these are external quality control (EQA) and internal quality control (IQC) [1]. IQC involved inter and intradepartmental seminars to improve the process, turnaround time, monitoring errors and troubleshooting on abnormal occurrences. EQA activities involve participation in external proficiency activities as well as bench marking with remote centres [1]. In America the association of Anatomic directors published three articles on quality control; these have shaped quality management in Anatomic pathology [2]. The first is concerned with internal quality assurance which emphasizes turnaround time and reliability of the diagnosis [2]. The second article was concerned with the standardization of the pathology report. The third article was centred on the standardization of the consultation in Anatomic pathology [2].

An erroneous laboratory result exposes a patient to harm [3]. Quality assurance activities must address the three phases of the histopathological test cycle; these are the preanalytical, analytical, and post analytical [4]. The preanalytical phase, is said to account for most of the errors [4-6], this why it is often taken seriously in the laboratory [7,8]. Subjectivity in histopathology and cytopathology reports makes instituting QC in these test difficult. To overcome this shortcoming, the bulk of laboratory QC activities centre on internal quality control [9,10]. In large laboratories, it may be technically impossible to peer review all results issued .An ingenious way one laboratory set out its QC was to set up an internal QC committee and peer review mechanism which selects through systematic random sampling 10% of the previous week reports for review [10].The peer review led to comments to be made on made 19.6% of the results [10]. Comments which range from microscopic description (4%) through macroscopic description (3.1%), to 0.3% incorrect results, examined all aspect of the test process [10]. They summarized the positive outcome of instituting IQC to include:1 Stimulus at both conscious and subconscious level to be always accurate;2 more frequent case consultation among pathologists;3 uniformity in diagnostic terminology, grading system and criteria among Pathologists;4 feed back to the scientific and technical staff in terms of the technical quality and productivity of the department, among others [10].

We present a snapshot review of an earlier participation in a multicentre lymphoma study, by evaluating the performance of our histology blocks as an external quality control for tissue biopsy. The focus of the laboratory is to establish an enduring quality management system. In the next articles we shall examine several aspects of internal quality controls as they exist before we institute a more robust quality management.

2. MATERIALS AND METHODS

A retrospective study of the performance of randomly selected formalin fixed paraffin embedded (FFPE) lymphoma tissue contributed to a Sub Saharan Africa lymphoma consortium study was carried out. Sixteen (16) FFPE tissue blocks from 14 lymphoma cases were contributed. Of the FFPE blocks selected two (2) were from 2007 collection, three from 2008 collection and the rest from 2009 collection. Four of the FFPE blocks from two patients diagnosed as human immunodeficiency virus (HIV)associated lymphoma followed a different analysis pathway and thus excluded from the study. The remaining 12 FFPE blocks were subjected to immunohistochemistry analysis and cores were taken for tissue microarray analysis. Those treated to immunohistochemistry analysis are included in the study while the tissue microarray processed cores are excluded from this study. The tissues were subjected to a panel of 40 antibodies. Feedback was sent to us as hard copies and electronic copies and comprised of general comments and results of immunohistochemical analysis. The data is fed into excel Microsoft statistical package for analysis.

3. RESULTS

Twelve FFPE lymphoma tissue from 5 females and 7 males were analysed. This is represented in a bar chart in Fig. 1.

The youngest patient was 3 years and the oldest 70 years, the range being 3 -70 years. This is represented in Table 1 and the bar chart in Fig. 2. The mean age of patients was 37, median age was 45 and modal age was 60 years.

Table 1. Shows the age distribution of patients in the study

Age groups	Frequency	Percentages
<21	5	42
21-30	0	0
31-40	1	8
41-50	1	8
51-60	4	34
61-70	1	8
Total	12	100

The EQA statement was that our tissues were not optimally fixed, and the ideal fixative neutral buffered formalin was not used. The unbuffered fixative employed in our centre is usually acidic and may have affected the antigen recovery in immunohistochemistry. That processing may have distorted the cellular morphology and the tissue fragility resulted in lack of adhesiveness to the glass slide. The immunohistochemistry result was affected by the acidic formalin with less avid

antibodies such as Ki67 and bcl16 staining poorly while CD20 staining was more reliable. The morphologic diagnosis from our centre was judged to be good.

A total of 40 antibody panels employed in lymphoma diagnosis were tested for each of the 12 lymphoma patients and this amounted to a total of 480 cells. Brittle tissue sections did not adhere to the glass slide and were marked as no core NC which otherwise known as non-conformities, and therefore no staining reaction was expected in such cells. The total number of NC recorded was 132.

Table 2. Showing distribution of cores

Total no of Cores That Fell off (NC)	Total No of Intact Cores	Total No of Cores
132	384	480

4. DISCUSSION

The performance of our tissue in this unintended EQA was an eye opener and opens the door for necessary improvement in our quality. A 26% fall off rate in our tissue cores in immunohistochemisistry is undesirable. The initial handling of a tissue in histopathology from the preanalytical phase to the analytic phase is of utmost importance [4,5,11].The common preanalytical errors in anatomic pathology are grouped into clinical failures
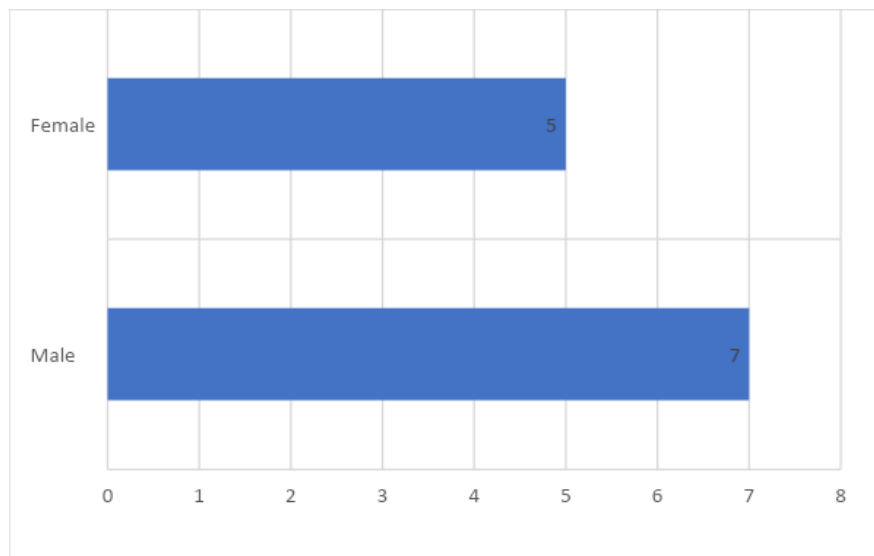

Fig. 1. Bar chart showing the sex distribution of patients from whom FFPE were selected for study

(wrong clinical procedure, inappropriate ordering, erroneous, incomplete or misleading clinical information) and specimen transportation and delivery(container mislabelling, wrong fixative and poor preservation [12]. The EQA identified use of wrong fixative type (non-use of neutral buffered formalin) and poor fixation as the preanalytical error that contributed to the non-conformity). It was suggested that the alternative constitution of formalin employed in our center leaves the tissue acidic and damages the tissue antigens thereby impacting negatively on subsequent immunohistochemistry results. The man handled tissue distorts a lot of information and the patient the worse for it, as immunohistochemistry results are employed in targeted therapy which may improve patient's prognosis.At the same time the need for pathological diagnosis is increasing as non-communicable diseases incidence is increasing in the developing countries [13]. This means the medical laboratories must brace up for this challenge by providing quality test results which will definitely improve patient care [13].

One of the comments the assessors made about some of the tissue blocks was that they were brittle which might have accounted for some of the lift off during staining. This calls to question some of our pre analytical aspects like long duration of tissue fixation and the use of fixatives containing mercuric chloride and alcohol, which normally makes the tissue brittle. But these fixatives were not employed in our laboratory,

however the duration of fixation in some instances is long, this can produce hardened brittle tissue upon processing. Some aspects of the analytical phase such as the non-use of histopathological range wax which melts at 56ºC and the poor temperature control at impregnation could produce brittle tissue. These might account for the brittle tissue and lift off which was experienced in some blocks during staining. The analytical phase of histopathology test in one systematic review accounted for between 4% and 42% errors in anatomical pathology tests in the studies reviewed [14].

The use of acidic formalin was suggested for the weak staining reaction to fragile antibodies like bcl6 and Ki67, while CD20 reaction was generally better. The causes of weak reaction at immunohistochemistry are grouped into three (1) low enzyme activity (2) insufficient antibody activity and (3) problem with tissue selection [15, 16]. The low enzyme activity might be due to buffer incompatibility or impaired enzyme substrate reaction. Among the numerous reasons for insufficient antibody activity, loss of antibody potency due to improper storage appears to be possible with our blocks. Our tissue blocks storage room is not particularly conducive. Perhaps temperature modification through installation of air conditioners might be of benefit, as average temperatures and humidity are in the range of 30ºC and 98% respectively all year round in Calabar. Some blocks
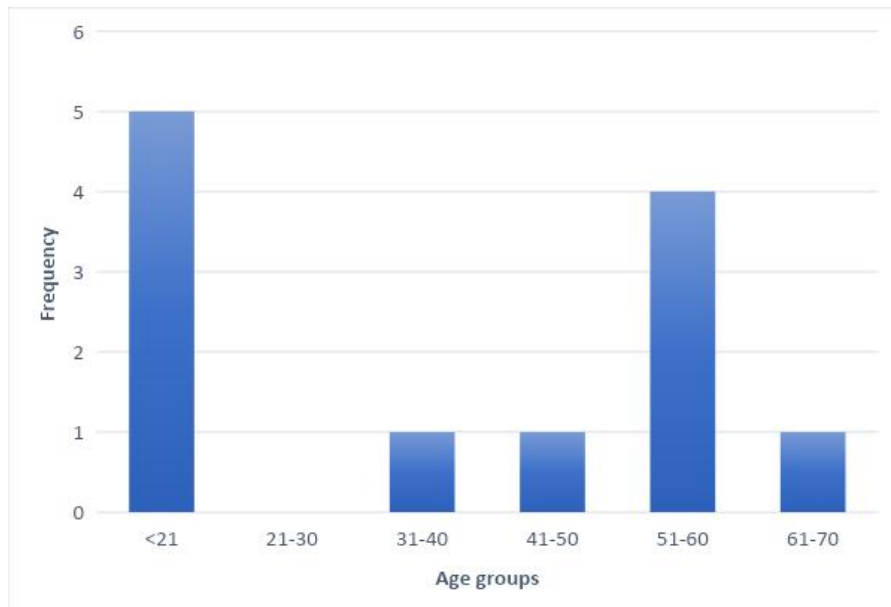

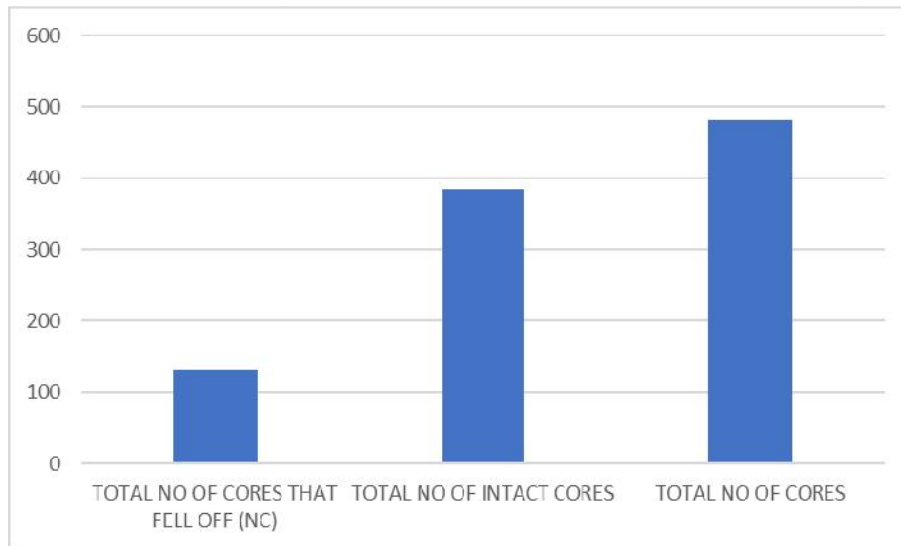**Fig. 2. Bar chart showing the age distribution of patients**

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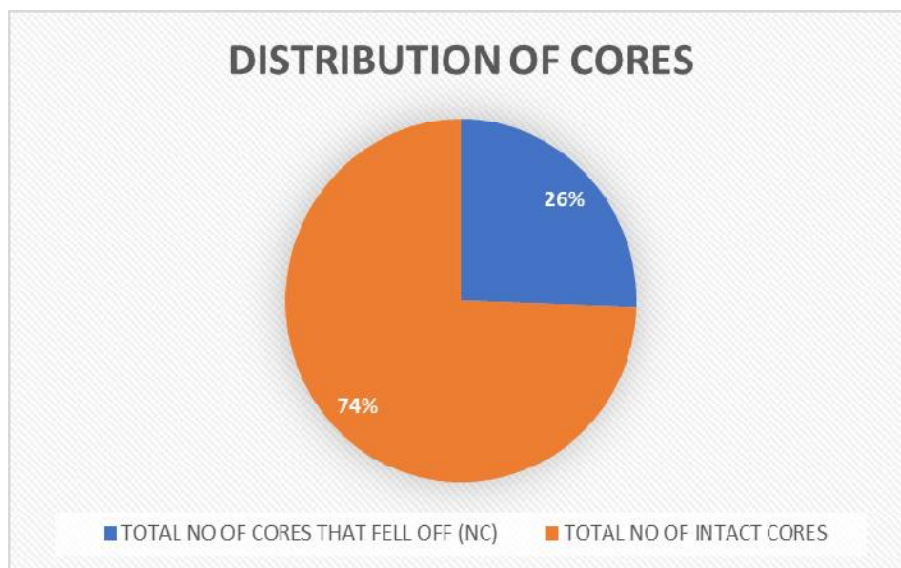**Fig. 3. Bar chart showing the behaviour of cores**

Fig. 4. Graphical representation of core distribution

become moldy which is damaging to the tissues. The problem of tissue selection as it might cause reduced staining could be as a result of insufficient deparaffinization, inadequate antigen retrieval or loss of signals over time.The dominant analytical errors in anatomical pathology test are instrument caused errors, poor techniques as well as errors of wrong interpretation [14]. The post analytical errors relate to result communication breakdown, poor communication within the laboratory and increases in turnaround time [5]. Anything in the histopathological test cycle that deviates from the normal with the potential of impacting on the patients care is called 'Non conformity'(NC)(4).In laboratories with well set up quality assurance and QC daily recordings of NC are kept and the manner in which such occurrences are resolved are also documented. In many countries with tight regulation of laboratory practice, such records are integral of accreditation enquiries [17].

Robust internal quality assurance measures will need to be put in place in our laboratory to improve the quality of our laboratory results. This

requires extreme commitment of time and resources, but this must be done for the sake of our patients. The method of continuous internal quality assurance which seeks to review 10% of the test result at every step of the test process seems the most feasible to adopt [10,11,18-20].In this cost conscious era and so as to satisfy accreditation bodies that are beginning to ask questions, the institution of QC and QA is the way forward [1,21,22]. Just as College of American Pathologist has driven QC and QA [23,24], in Nigeria College of Nigerian Pathologist and the Federal ministry of health are beginning to drive the process. In conclusion, a good laboratory test result must be accurate, timely, reliable and must satisfy the client. It does appear that until we improve our process the test results are not very reliable.

5. CONCLUSION

The quality of histopathology biopsy reports from our department needs improvement. This can be achieved through the institution of continuous internal quality control and other quality assurance measures.

6. RECOMMENDATIONS

The recommendation of the author is the immediate institution of continuous internal quality control, as well as external quality control participation. Management of the hospital and laboratory should support the laboratory with resources to enable it achieve these goals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was granted by the institutional ethical review board.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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