Original Article

Derivation of a List of Priority Antibodies from the Analysis of a Cohort of Cases sent from Nigeria for External Consultation

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Abstract

Introduction: The major obstacle to developing sustainable services in a resource-limited setting, which hitherto has not had routine availability of diagnostic immunohistochemistry (IHC), is choosing from a large array of over 200 antibodies that are currently in use in the Western world. **Materials and Methods:** By critically analyzing the use of diagnostic IHC in a cohort of 360 cases sent for consultation from Nigeria to the UK between January 2014 and May 2016, we have derived a list of antibodies that could meet over 85% of current diagnostic IHC needs in Nigeria. **Results:** From our analysis, a starter list of only two antibodies could immediately meet over 30% of the IHC needs. Having mastered this starter list, the service could move to the next step by adding 23 other antibodies which could meet another 85% of diagnostic IHC needs. **Conclusions:** Testing with these 25 antibodies can be done at least twice weekly to address the 3 areas mentioned above and greatly increase the chances of success in establishing a sustainable service. We recommend this list to the various groups working with diagnostic IHC in Nigeria and look forward to reports of their efforts.

Keywords: Antibodies, immunohistochemistry, Nigeria, priority list

Introduction

The quality of healthcare in a country is only as good as its pathology and laboratory services, and this cannot be sustainably provided from outside the country.[1] Inadequate pathology services lead to a cycle of ineffective healthcare knowledge, research, and practice.[2] The African Strategies for Advancing Pathology Group Members believe that it is possible to provide accurate and cost-effective pathology diagnostic testing for noncommunicable diseases by simply improving and extending existing laboratory services.[3]

In recent times, Nigeria has progressed quite remarkably on the road to widespread availability of quality pathology services. Just over a decade ago, diagnostic histopathology was available mainly in a few academic medical centers (teaching hospitals for some medical schools).[4] Now pathology services at the level of the basic hematoxylin and eosin stains can be found in virtually all the 36 state capitals, in general hospitals of some other major towns and in some established private laboratories in the major cities all over the country. The current situation as revealed by a recent survey[5] indicating that Nigeria now has one pathologist to just over a million population (and about eight histo/cytotechnologist to one pathologist) may appear dismal; however, this is a significant improvement from the ratio of one pathologist to 3 million population less than a decade ago.[4] It is worth noting that because Nigeria has an in-country system that produces these trained professionals, their ratio to the Nigerian population is moving quickly in the right direction in a sustainable manner. However, there may be need to pay attention to ensuring that posts/jobs are created quickly enough to absorb them as they are produced. There is also the need for sustainable continuing professional development and quality assurance schemes to become available within the country. At this time, this is a work in progress.

The next major step for further progress to be achieved is for diagnostic immunohistochemistry (IHC) to become routinely

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available to the Nigerian histopathologist. Services that can be accepted as routine IHC, by the standards used in developed countries, are found only in South Africa.[5] In spite of a progressively increased awareness of its importance over the past decade, IHC for diagnostic purposes is not yet routinely available to the average Nigerian histopathologist who often has to send cases abroad.[6] This is by no means due to a lack of effort on the part of various individuals and groups. We are aware of many different efforts that have either failed completely or achieved only very limited success. It appears to us that the major difficulties to introducing an IHC service in Nigeria can be broadly categorized into three as follows:

1. Economic viability – without state support or widespread health insurance, funding of healthcare in Nigeria is largely on pay-per-service terms for individual patients. Limiting waste by ensuring that there is sufficient demand for antibodies in stock is critical
2. Technical competence – mastery of the intricacies involved in getting a good staining result with each antibody depends to a great extent on repeated practice and use.[7] The frequency of staining runs for each antibody is therefore vital
3. Interpretational proficiency – familiarization of pathologists with the staining characteristics of each antibody requires frequent use in real-clinical scenarios.

In this article, we propose that these difficulties can be overcome using a limited number of antibodies which largely meets current service needs (and hence generates income). This small list can be used frequently enough so that technical competence and interpretational proficiency are gained in a timely manner.

Materials and Methods

Over a period of 12 years, the authors, most of who practice in Nigeria, have largely met their need for diagnostic IHC by sending cases in consultation to the lead author in the United Kingdom. The computer database records (at the receiving laboratory) and reports for a cohort of cases received between January 2014 and May 2016 were analyzed to determine which antibodies were used in the process of reaching a final diagnosis. The antibodies used were separated into two groups based on how frequently they were used in this cohort of cases. Each antibody in each group was then further considered to see whether it should be included in a shortlist of antibodies.

Results

A total of 360 cases were received in the study. A little over one-third of these (124 or 34.40%) were cases of breast carcinoma sent for estrogen receptor (ER) and human epidermal growth factor receptor 2 (Her2) assessment. Out of the remaining 236 which were sent for diagnostic assessment, a final diagnosis was made in 41 cases (11.3% of the total and 17.4% of the diagnostic cases) without using IHC [Figure 1]. A total of 92 different antibodies were utilized in the diagnostic assessment of the 195 cases in which IHC was required. The top 24 most frequently used antibodies are listed in Table 1, and all the others are listed in Table 2.

A short list of antibodies was derived as follows: each of the top 24 antibodies was considered individually in the light of their purpose in the diagnostic scenarios to see whether it was possible to dispense with them or substitute them with other antibodies in the top 24. The three antibodies highlighted in red were considered dispensable as follows:

1. Epstein-Barr virus-latent membrane protein1 while being informative was not considered critical for reaching the diagnosis in any of the 19 cases for which it was used
2. High-molecular-weight cytokeratin (CK) was used to delineate the basal/myoepithelial layer in breast and

Table 1: The top 24 antibodies by frequency

Antibody	Number of times used
Ki-67	83
AE 1/3	72
S-100	65
Desmin	51
CD20	44
CD5	42
CD34	41
TTF-1	33
CK7	33
CD-117	32
Bcl-2	32
CD45	32
CD30	30
CK20	30
CD10	27
EMA	24
P63	23
CD56	23
DOG-1	22
WT-1	21
EBV-LMP1	19
CD3	19
HMWCK	19
CD23	16

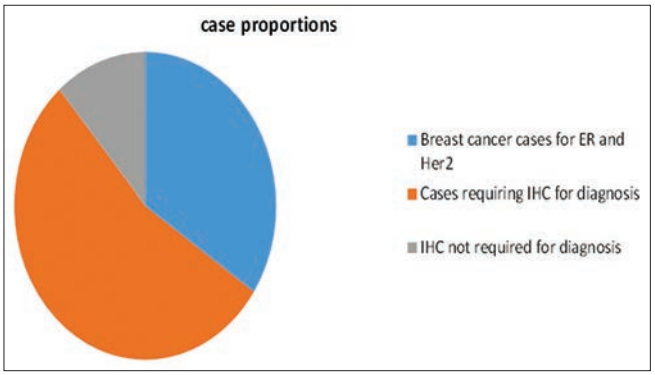

Figure 1: Cases divided into broad groups according to need for immunohistochemistry

Table 2: All other antibodies

Antibody	Number of times used	Antibody	Number of times used
SMA	15	CD31	4
VIMENTIN	15	BER-EP4	4
PSA	14	CD4	4
P504S	13	PSAP	4
CDX2	13	AFP	4
p16	12	Oct-04	3
TdT	12	CD8	3
CALRETININ	11	Glycophorin C	3
CD15	11	RCC	3
KAPPA	11	THYROGLOBULIN	3
SYNAPTO	11	CALCITONIN	3
LAMBDA	11	SMMHC	3
Cyclin D1	11	CK5/6	2
CHROMOGR	10	KSHV	2
PAX5	10	CD61	2
NSE	9	COLL 4	2
CD68	9	CAM5.2	2
MUM-1	9	HCG	2
Melan A	9	SARCOMERIC ACTIN	2
CD21	7	CK19	1
ALK-1	7	CA125	1
HEPPAR	7	P24	1
CD99	7	GFAP	1
HMB45	7	IDH1	1
INHIBIN	6	CD7	1
CD79a	6	CD43	1
PLAP	6	CK14	1
NFP	6	B-CATENIN	1
CD38	5	ER	1
CD138	5	CEA	1
P53	5	CALPONIN	1
GCDFP	5	Mammoglobin	1
BCL-6	5	P40	1

prostate biopsies; a function which could adequately be performed by p63

3. CD3 was found to be critical for diagnosis in only 4 cases: 1 case of enteropathy-associated T-cell lymphoma, 1 case of precursor T-cell lymphoblastic lymphoma, and 2 cases of mycosis fungoides.

The antibodies in Table 2 were also analyzed to see the impact of not including them in the shortlist. At the end of the analysis, two antibodies (highlighted in blue) were included in the shortlist as follows:

1. Prostate-specific antigen (PSA) was considered critical in diagnosing three cases of metastatic prostatic carcinoma and was vital as part of a panel to assess many other cases of metastatic adenocarcinoma along with CK7, CK20, CDX-2, and thyroid transcription factor (TTF-1)
2. CDX-2 was considered critical in diagnosing 5 cases of metastatic gastrointestinal tract carcinoma and was vital as part of a panel to assess many other cases of metastatic adenocarcinoma alongside CK7, CK20, PSA, and TTF-1.

Table 3 displays the impact of excluding 22 antibodies from the shortlist. All other excluded antibodies were not considered to have any significant impact on ability to conclude diagnostic work on the 195 cases.

In summary, adopting a shortlist of 23 antibodies (other than ER and Her2) as shown in Table 4 would have meant that 24 out of the 195 cases needing IHC for diagnostic assessment could not have been concluded. In other words, these 23 antibodies meet the diagnostic IHC needs of 87.7% of cases in this cohort (excluding the breast cancer cases and cases that did not require IHC for diagnosis).

The list of antibodies shown in Table 4 is organized to indicate panels (of typical clinical scenarios) in which they may be used. Each of the antibodies of course has various other uses outside the panels indicated depending on the context, for example, p63 may be useful to indicate squamous differentiation, CD34 may be used as an endothelial marker, CD117 as a myeloid marker, WT-1 to mark mesothelium, CD10 to mark endometrial stroma or classical renal cell carcinoma, cytoplasmic TTF-1 to mark hepatocellular differentiation, CD5 to indicate malignancy in thymoma, and CD56 to support the impression of neuroendocrine differentiation, etc.

Discussion

For decades now, IHC has been critical to quality diagnostic histopathology. Yet, while the advanced world is moving beyond IHC to other molecular methods as part of the tools available for routine diagnostic histopathology, most pathologists in Africa in general and Nigeria, in particular, are yet to benefit from the routine availability of IHC for their diagnostic work.

Nigerian pathologists have largely become aware of the place of IHC in their work by reading literature, going for attachments overseas, and attending courses both locally and overseas. Some have been privileged to have limited access to IHC by virtue of participation in well-funded major research projects with international partners.[8] Some of these research projects set up IHC facilities within the country, and it could have been hoped that those would transform into routine service laboratories. However, more often than not, these advanced facilities have either folded up shortly after the conclusion of the research project or failed to extend the IHC facilities beyond the large academic medical center in which the research project was domiciled. This type of occurrences is recurrent for Africa, and some believe that it is related to the way in which global health initiatives and international research projects are structured around specific diseases, creating disease silos that are in competition with one another for attention and funds to the ultimate detriment of the people whom they purport to help.[9] Perhaps, serious attention needs to be paid to a proposal for some form of a tax on funding for disease-specific research projects in resource-poor settings that would be spent on general/country-wide improvement of pathology and laboratory services that is not particularly related to that disease.[10]

Table 3: Impact (in terms of cases that could not have been concluded) of excluding some 22 antibodies from our shortlist

Excluded antibodies	Impact (cases that could not have been concluded)
Vimentin	1 case of desmoplastic small round-cell tumor*
	1 case of metastatic renal-cell carcinoma
TdT	1 case of Type A thymoma
	1 case of precursor T-cell lymphoblastic lymphoma
Calretinin	1 case of adrenal cortical carcinoma*
Kappa and lambda	1 case of plasmacytoma
Chromogranin	1 case of adrenal cortical carcinoma*
	1 case of extradrenal paraganglioma
NSE	1 case of desmoplastic small round-cell tumour*
Melan A	1 case of adrenal cortical carcinoma*
ALK-1	1 case of anaplastic large-cell lymphoma
HEPPA-1	3 cases of hepatocellular carcinoma (cytoplasmic staining with TTF-1 could been a substitute)
	1 case of intrahepatic cholangiocarcinoma
CD99	2 cases of Ewing sarcoma PNET
Inhibin	1 case of adrenal cortical carcinoma*
PLAP	1 case of yolk sac tumour*
NFP	1 case of gliosarcoma*
AFP	1 case of yolk sac tumour*
Glycophorin C	1 case of leukemoid reaction in bone marrow trephine
KSHSV	1 case of Kaposi sarcoma
HCG	1 case of yolk sac tumor*
p24	1 case of HIV-associated Mikulicz tumor
GFAP	1 case of gliosarcoma*
IDH1	1 case of gliosarcoma*
CD163	1 case of histiocytic sarcoma

*Same case. PNET: Primitive neuroectodermal tumor

Table 4: Final shortlist of 25 antibodies and typical scenarios (panels) in which they may be used

Antibody	Typical panel
ER	Breast cancer panel
HER2	
Ki-67	Non-Hodgkin's lymphoma panel
CD20	
CD5	
CD23	
CD10	
Bcl-2	
CD30	Hodgkin's lymphoma panel
CD45	
CD30	Undifferentiated tumor panel - Possibly Lymphoma, carcinoma, melanoma, sarcoma, germ cell tumor
CD45	
EMA	
Desmin	
S-100	
AE1/3	
CD56	
p63	Basal/myoepithelial layer marker for prostate and breast
CK7	Metastatic carcinoma panel
CK20	
TTF-1	
WT-1	
CDX-2	
PSA	
CD34	GIST panel
CD117	
DOG-1	

GIST: Gastrointestinal stromal tumor, ER: Estrogen receptor, Her2: Human epidermal growth factor receptor 2, CK: Cytokeratin PSA: Prostate specific antigen

In recent times, clinicians and patients in Nigeria have increasingly become aware of the benefits of IHC for histopathologic diagnosis. This awareness has created a rising demand which at present is largely met by sending paraffin wax blocks overseas as part of diagnostic consults/second opinion requests. Many efforts to setup diagnostic IHC services in Nigeria have been far less successful than was anticipated.

We believe that the main obstacles to the establishment of routine diagnostic IHC services in the country can be placed into three main categories.

Economic viability

Unlike in advanced economies where health-care costs are met by insurance schemes or funding from general taxation, health-care costs in Nigeria are met predominantly on a pay for service basis by the patient. This means that in both absolute and relative terms, there is very little money available for health-care services. With respect to IHC which is relatively quite expensive, especially at the rather low volumes of use as obtains in Nigeria, Nigerian pathologists have unfortunately been guided into emulating their foreign counterparts in latter's liberal use of antibodies. Compared to their Nigerian counterpart, the typical American or European pathologist is not so cost-constrained and so, is not overly concerned about whether each individual antibody he requests is absolutely necessary. Indeed, it is very common and often perceived wisdom to use multiple antibodies in a panel for the same specific purpose while working on a case. In the experience of the authors, many efforts at setting up a diagnostic/clinical IHC service in Nigeria have been characterized by an inordinately long list of antibodies which often expire before use and cannot be replaced once the initial start-up grant is exhausted. Owing to their limited health-care resources, developing countries cannot afford the models used in developed countries.[11] Conventional procedures may need to be supplemented or supplanted by different approaches that are more suited to the local conditions.[12] Even if the new approaches adopted are less than perfect, surely it must be ethical to improve a bad situation substantially in any way that is possible at the time, pending the attainment of the ideal.[13]

Technical competence

A key part of a diagnostic IHC service is the ability to consistently and reproducibly carry out the staining procedure. While there is a lot of the process that is common for all antibodies, each antibody does need to

be mastered by the medical laboratory scientists in terms of optimization, validation, troubleshooting, and ongoing quality control/monitoring.[7] Even in resource-rich settings, there can be considerable difficulties with interlaboratory reproducibility for some antibodies.[14] Efforts to setup a service which do not take this into account find that the technical staff is overwhelmed when they have a large number of different antibodies to deal with. This is especially so in view of their inexperience with IHC in general, and the fact that the conditions (such as ambient room temperature and availability of deionized water) are so very different from what the inevitably foreign antibody manufacturers assumed would be the case. There is, therefore, an obvious advantage in having a very limited repertoire at the beginning.

Interpretational proficiency

As everyone who uses IHC routinely knows, there is a lot of nuance in the interpretation of staining reactions for various antibodies. Lack of experience can all too often result in a diagnostic pathologist being misled by some immunohistochemical reactions. Having a large repertoire of antibodies which are not used frequently may mean that the Nigerian pathologist never quite gets a grip on how best to use IHC, therefore, running a significant risk of overall poor results.

It is our belief from experience that the use of a limited antibody list which can be run as frequently as possible (at least twice a week) will address these issues and greatly increase the chances of success in establishing a diagnostic IHC service in Nigeria or similar resource-limited environments. This approach of using a limited panel/list of antibodies to achieve diagnosis in a majority but not all cases is one that has been advocated for specific diseases.[15]

This study uses a cohort of cases where demand for IHC by Nigerian patients mainly located in the commercial capital of Lagos was met by sending paraffin wax blocks to a pathologist working in a histopathology laboratory in the United Kingdom. Giving due consideration to the fact patients in resource-limited settings have to pay out of pocket for the antibodies required for a case engenders a more frugal approach to the use of antibodies than what obtains in the developed world. This analysis was intended to see how much could have been achieved if experienced pathologists working in Nigeria had access to good quality IHC albeit with a limited stock list of antibodies.

Based on this analysis, with a starter panel of only two antibodies (ER and Her2) for breast cancer, a setting in Nigeria could soon reduce its reliance on overseas consults by over 30%. There would, of course, be a continuing need for overseas support with Her2 *in situ* hybridization for the indeterminate cases. Success at this stage would give such a setting, the confidence and experience required to progress to the next stage of trying to master the use of 23 other antibodies on our short list. Our analysis shows that success at this stage could produce a further reduction in reliance on overseas consults of over 85%. No doubt more antibodies could be added after this, but perhaps most importantly, the Nigerian histopathologist would have learned a more frugal approach that is more suitable to the circumstances and economy in which she practices.

With respect to the issue of quality control in IHC, our view from experience is that premature attempts to join foreign external quality assurance schemes (which are not geared to serve the stage at which emergent Nigerian services are) are counterproductive. Until a formal technical external quality assessment scheme which is tailored to meet the needs of Nigerian IHC services is set up (sooner rather than later), informal quality assurance relationships with designated foreign laboratories may have to serve the purpose. Studies have shown that remote monitoring, feedback, and audits can support quality for laboratories in low-resource settings which lack strong regulatory support for laboratory quality.[16] Similarly, Nigerian histopathologists may need to establish mentorship relationships with more experienced overseas-based pathologists, as they work through the process of achieving interpretational proficiency.[17]

Another issue that a start-up effort for IHC in Nigeria and similar resource-limited settings must consider is the place/value of automation in IHC. In contrast to automation, manual processing offers flexibility that may be critical for start-up efforts in a setting in which conditions differ markedly from what the antibody manufacturers expect. Furthermore, jumping to automation without a passage through manual may result in a knowledge gap for technical staff that reduces the ability to troubleshoot when things go wrong in the machine.[18]

Conclusion

We have identified key stumbling blocks to establishment of diagnostic IHC services in Nigeria. We propose that these can be overcome using a priority list of antibodies when trying to setup such a service and we have derived a shortlist of 25 antibodies. We recommend that all the antibodies are run at least twice a week to facilitate the achievement of technical competence and interpretational proficiency, all in the context of economic viability which is related to demand. Quality assurance may have to be on informal bases at the outset, utilizing the support of overseas colleagues and laboratories. We recognize that this study has a few limitations which include the moderate numbers of cases and the fact, they were all reported in one UK laboratory (and mainly by one pathologist) which might have had an impact on the choice of antibodies, we, however, hope that some teams will attempt to follow our recommendations and report the outcome of their efforts in the near future.

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Conflicts of interest

There are no conflicts of interest.

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