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ORIGINAL ARTICLE

Immunohistochemistry usage profile in a Malaysian tertiary hospital's histopathology laboratory

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Abstract

Introduction: Immunohistochemistry (IHC) was commenced in 1986 at the Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur and its usage has grown for the past 30 over years, hence it was felt that a review was timely in view of the scarcity of literature on IHC usage. *Materials and Methods:* All cases received by the Department of Pathology for histopathological examination between 1 July 2018 and 30 June 2019 were retrieved from the Laboratory Information System (LIS). All the IHC requests over this period were tabulated, with the exception of renal, muscle, rectal and nerve biopsies with their pre-defined algorithms for stains and cytological specimens. IHC stains performed solely for purpose of directing targeted treatment were also not included. *Results:* Immunohistochemistry was performed in 2044 (21.1%) of the total of 9686 cases, with a total of 5969 IHC stains performed i.e. 2.9 (5969/2044) IHC stains per case. All 91 antibodies available were used at least once during the study. 14 histopathologists (5 with < 10-years and 9 with ≥ 10-years postgraduate specialist experience) reported on the cases with no significant difference (p=0.90) in their usage of IHC stains. Among the most common IHC stains used, requests for Ki67 and MNF116 showed higher standard deviations compared with p63, CK7 and S100 among the histopathologists. From the relatively higher standard deviation for Ki67 and MNF116 it appeared that there was a greater difference in the requesting pattern between histopathologists for these two antibodies. *Conclusion:* The rate of use of IHC in our centre seems compatible with that of an academic centre. Personal preferences of the histopathologists, rather than years of postgraduate specialist experience appeared to influence the rate of usage and choice of antibodies.

Keywords: Immunohistochemistry, usage, histopathologists, histopathology laboratory

INTRODUCTION

Immunohistochemistry (IHC) has slowly entrenched itself into the histopathology practice over the past 3 decades and is now considered almost integral in routine histopathology diagnostic work. Undoubtedly, it has wide ranging utility, including providing more specific classification of tumours, determination of the primary site of metastases and more recently serving as prognostic and predictive biomarkers.[1,2] Yet, despite its important role, while IHC seems a commonality in developed and many developing countries, it still remains unavailable in less developed ones. For example up to 2017, only half of 16 medical centres which were part of established cancer consortia in Nigeria had IHC facilities.[3] This is a marked difference from what was observed in the United Kingdom, which by the mid-1980's had 71% and 78% of laboratories reported to be utilising IHC.[4,5] Although IHC has been an established technology at the Department of Pathology, University of Malaya Medical Centre (UMMC), Kuala Lumpur since 1975 with immunofluorescence being first introduced for study of immune complex deposition in fresh renal biopsy tissues, it was only in 1986 that IHC on the routine formalin-fixed, paraffin-embedded tissue was commenced. It resulted in the Department of Pathology at UMMC being the first centre in Malaysia to formally introduce IHC into the routine diagnostic histopathology laboratory service and this paved the way towards a rapid propagation of the technology throughout the country. From the few historical landmark markers which broadly differentiated

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carcinomas from sarcomas and lymphomas, immunohistochemical markers have evolved from being aids to diagnostic differentiation of tumours to assume roles as sophisticated surrogate markers of distinctive genomic pathways, several being commonly utilised as predictive biomarkers for targeted treatment e.g. the human epidermal growth factor receptor 2 (HER2) amplification in breast and gastric cancers[6,7], to the identification of rearrangement of the anaplastic lymphoma kinase (ALK) gene[8] and c-ros oncogene 1 (ROS1)[9] in lung cancers. The armamentarium of immunohistochemical markers continues to grow each day with most markers being commercially and readily available. This prolific expansion is however not without some concomitant drawbacks and also pose concerns of cost escalation in producing a histopathology report today.

Since its implementation, there has been no review of the immunohistochemistry service at our centre and it was felt that it would be appropriate to do so with regards to understanding (1) the percentage of histopathological specimens that are subject to IHC in its present stage of evolution and (2) the antibodies which are most frequently requested. Thirdly, that while IHC is generally acknowledged as an important aid in routine diagnostic histopathology, there is very little that determines an individual histopathologist's usage by way of established guidelines. Variations are therefore expected among histopathologists in their choice of antibodies as well as the frequency they embrace IHC to assist them in their practice. It is anticipated that years of experience and familiarity with the subject matter will influence the histopathologist's practice and dependence on adjunctive aids. This was demonstrated by Plourde *et al*. with genitourinary pathologists ordering less IHC stains in their workup of extended core prostatic biopsies compared with non-genitourinary-specialised pathologists.[10] We were therefore interested to determine the IHC requesting pattern of histopathologists at our centre. At the Department of Pathology, UMMC all the histopathologists are randomly rostered to report the cases which are received by the department without matching of type of case by organ system or provisional clinical diagnosis to any specific histopathologist. Although, certain histopathologists have interest in special areas, the sign-outs are by whoever is scheduled for reporting duty, even though the histopathologists are free to consult those with speciality interest, whenever they see a need. Thus, it would appear that in our case, years of general experience of the histopathologists, rather than an especial familiarity in a particular area, may influence the usage of IHC in their work. Hence, we chose to classify this "general experience" based on the years of postgraduate specialist qualification of the histopathologists involved in the study, without further address of the speciality interest of the individual histopathologists. The findings of this study must also take cognisance that the University of Malaya Medical Centre is a tertiary public hospital which also serves as a teaching and training facility for the University of Malaya Medical Faculty. The clinical service of the Department of Pathology has therefore to be viewed in the context of this dual functionality.

MATERIALS AND METHODS

All cases received from UMMC patients by the Histopathology Division of the Department of Pathology for the period between 1 July 2018 and 30 June 2019 were retrieved from the Laboratory Information System (LIS). All the IHC requests made during this period were similarly retrieved and tabulated. The antibody requested, the number of IHC stains requested for each case and the respective histopathologist making the request were also enumerated. "Special" specimens such as renal, muscle, rectal and nerve biopsies with their pre-defined algorithms for IHC stains and cytological specimens were excluded from the total case load and from the study. Except for ER, PR and HER2 which apart from guiding treatment were used for subtyping breast cancers, IHC stains performed solely for the purpose of directing targeted treatment were also not included in this study.

The study was approved by the Institutional Review Board (IRB) of the University of Malaya Medical Centre (MREC ID NO: 202017-8144) and carried out in compliance with the Declaration of Helsinki. All patient identification was anonymised and the information obtained for this study was only made available to the authors, ensuring protection of data confidentiality.

Statistical analysis was carried out using SPSS version 24.0 (IBM, Chicago, Illinois, USA). Statistical significance was set as $p < 0.05$.

RESULTS

A total of 9686 cases which satisfied the inclusion criteria were received at the Histopathology Division of the Department of Pathology for the

period between 1 July 2018 and 30 June 2019. Immunohistochemistry was performed in 2044 (21.1%) cases, with a total of 5969 IHC stains performed for all these cases, which averaged to 2.9 IHC stains requested per case in which IHC was carried out.

Ninety-one antibodies were available for use during the period of study and all the antibodies were used at least once. Figure 1 demonstrates the 30 most frequently requested antibodies i.e. Ki67, ER, MNF116, PR, HER2, p63, CK7, S100, TTF1, CD3, CD20, CK20, SMA, CD34, CD10, CK5/6, EMA, CD68, desmin, vimentin, synaptophysin, chromogranin, CD31, AE1/AE3, GFAP, BCl2, e-cadherin, CD117, CD45 and CMV during the period of study.

Fourteen histopathologists reported on the cases during this period. The number of years of postgraduate specialist qualification of the 14 histopathologists ranged between 1-39 years

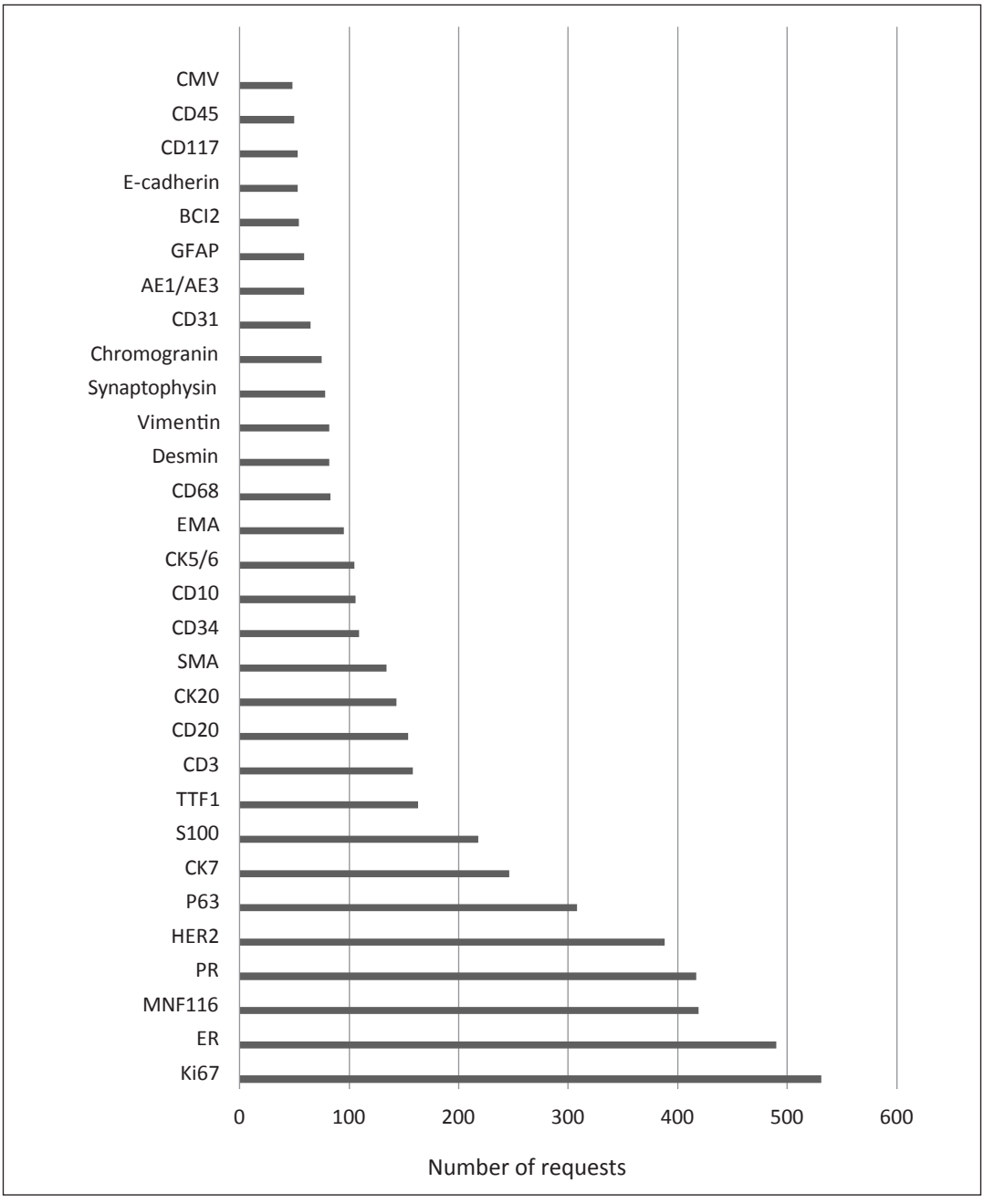

FIG. 1: Thirty most frequently requested antibodies

Table 1: Requests for immunohistochemical (IHC) stains as percentage of the histopathologists' caseload according to years of postgraduate specialist experience

Percentage of cases in which IHC was requested	<10 years specialist postgraduate experience	≥10 years specialist postgraduate experience	
Range	15.4-22.8%	14.0-34.1%	p=0.90
Mean±SD	18.6±2.7%	20.2±6.7%	

(mean=18 years). Postgraduate experience of ≥10-years was arbitrarily set as the cut-off between the histopathologists. 5 of the histopathologists had less than 10-years of experience and 9 had 10-years or more postgraduate specialist experience in histopathology reporting. IHC requests per case load of the individual histopathologists ranged between 14.0% - 34.1% (mean =19.6%). No significant difference (p=0.90) occurred in the IHC requests between histopathologists with ≥10-years (range=14.0-34.1%, mean±SD=20.2±6.7%) and those with <10-years postgraduate experience (range=15.4-22.8%; mean±SD =18.6±2.7%) (Table 1).

Although no significant difference occurred in the usage of IHC stains between the histopathologists with ≥10-years postgraduate experience and those with <10-years, it was noted that there was a higher standard deviation in the former group. Discounting ER, PR and HER2 which were prescribed antibodies in the workup of breast cancers in the majority of cases, we also proceeded to analyse the usage of antibodies by individual histopathologists to determine whether there was any individual preference for particular antibodies. This was limited to those antibodies which had ≥200 requests during the period of study. Table 2 shows the requesting pattern of Ki67, MNF116, p63, CK7 and S100 protein of the histopathologists. Requests for Ki67 among the histopathologists ranged from 1.8-19.6% of their cases (mean±SD=4.3±4.6%); MNF116 from 0-15.2% (mean±SD=3.4±3.7%); p63 from 1.0-5.9% (mean±SD=2.8±1.3%); CK7 from 1.5-5.7% (mean±SD=2.7±1.4%) and S100 from 0.6-3.4% (mean±SD=2.1±0.7%). From the relatively higher standard deviation for Ki67 (4.6%) and MNF116 (3.7%) it appeared that there was some difference in the requesting pattern between histopathologists for these two antibodies, more than for p63, CK7 and S100 protein, the standard deviations of which ranged from 0.7-1.4%. Fig. 2 illustrates that while most of the histopathologists, both those with ≥10-years and <10-years postgraduate specialist experience, did not vary much in their requesting pattern of the 5 aforementioned antibodies, one of the histopathologists with ≥10-years' experience demonstrated an inclination for use of Ki67 and MNF116.

DISCUSSION

Literature remains fairly scanty regarding the rate of IHC usage in histopathology laboratories although this technology and diagnostic aid has been used in routine histopathology for almost 4 decades. Immunohistochemistry was performed in 21.1% of cases at our centre, which seems a fair rate when compared with those noted by Shah *et al*.[1] In their comparison between

Table 2: Requests for Ki67, MNF116, p63, CK7 and S100 protein as percentage of the histopathologist's caseload

Histopathological cases with immunohistochemistry requests		
Antibody	Range (%)	Mean±SD (%)
Ki67	1.8-19.6	4.3±4.6
MNF116	0-15.2	3.4±3.7
p63	1.0-5.9	2.8±1.3
CK7	1.5-5.7%	2.7±1.4%
S100	0.6-3.4%	2.1±0.7%

histopathologists working at an academic centre and those outside of academia, they observed that the former used IHC in 11% and the latter in 26% for diagnosing a set of 200 carcinomas; the majority of the 200 cases studied having being referred to the academic centre for further management. It is noteworthy that in Shah *et al*.'s study, the actual diagnoses of the cases, except that they were invasive cancers, were not known to the academic histopathologists. The academic histopathologists were only provided with the haematoxylin and eosin (H+E) stained slides of the cases, age and gender of the patients, locations of the biopsied specimens (most of the referred specimens being biopsies) and the brief clinical histories as were available on the surgical reports of the referring histopathologists from the non-academic centres. The histopathologists in the academic centre reviewed the cases based on the H+E slides then listed the IHC stains they would request for each of the cases, but did not actually re-sign out the cases. Considering the above, the rate of use of IHC in our centre, albeit an academic centre, may differ slightly from that observed among the academic histopathologists in Shah *et al*.'s study and may be more similar to that observed among the non-academic histopathologists by virtue of the fact that in

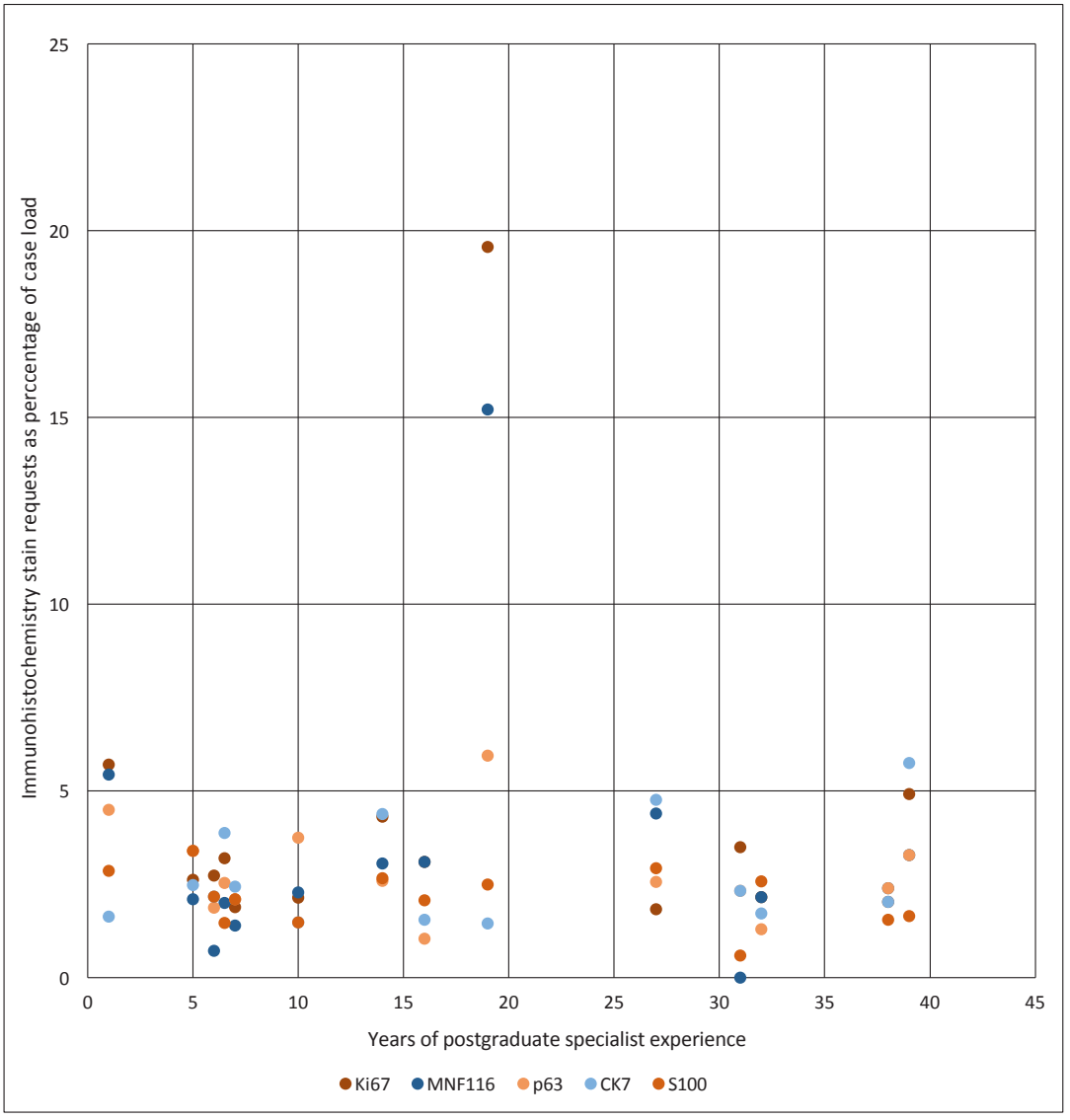

FIG. 2: Immunohistochemistry stain (Ki67, MNF116, p63, CK7 and S100) requests as percentage of case load versus the years of postgraduate specialist experience of the histopathologists

our study, the study histopathologists were making the primary diagnoses. At least for 2 reasons, one of speeding up workup of cases, histopathologists may order more IHC stains than required to clinch the diagnosis for each case, which could have happened in our centre. Secondly, the responsibility of an actual sign-out may influence histopathologists to be more cautious in ruling out mimics via IHC than in a simulated situation.

The number of IHC stains used in Shah *et al*.'s study was 3-4/case, with 3 for histopathologists in academia and 4 for those outside academia. In our centre, on an average 2.9 stains were used per case for the cases in which IHC was requested. This seems very similar to the rate used by the histopathologists practising in an academic centre in Shah et al's study. In the present study, we should be cognisant that unlike the histopathologists in Shah *et al*.'s study who worked on known carcinomas, all cases which satisfied the inclusion criteria, and which included non-malignancies, were enumerated. Like Shah *et al*., we did not find any significant difference in the rate of IHC usage between the histopathologists who were more and those who were less experienced. It is interesting to note that the academic histopathologists, both in Shah *et al*.'s study and ours, used slightly less number of IHC stains per case to aid their diagnoses than those from outside academia. The Department of Pathology, UMMC is training ground for future specialist histopathologists in the country and perhaps this intrinsically makes the academic histopathologists more disposed towards detailed study of the H+E stained section and placing less reliance on IHC for diagnostic purposes. Nevertheless, this hypothesis remains to be proven in the Malaysian setting and would need a proper comparison of IHC usage in non-academic with academic institutions.

While there was comparable rate of usage of IHC amongst pathologists with ≥ 10 years and those < 10 years, which then seems to discount experience as an influence, it was interesting to note that the standard deviation was higher in the former group, 6.7% compared with 2.7% in the latter. As the histopathologists were randomly rostered to report and sign-out the cases which were received by the department without matching of type of case to any specific histopathologist, it was assumed that the histopathologists' personal preferences were likely to have played a role in the usage of IHC stains in deriving the histopathological diagnosis. It was also interesting that among the antibodies which had ≥200 requests during the period of study, the usage pattern of MNF116 and Ki67 also showed a higher standard deviation as compared to p63, CK7 and S100 among the histopathologists. It was further noted that one of the more senior histopathologists (≥ 10 years of postgraduate specialist experience) demonstrated a preference for use of MNF116 and Ki67 in comparison with the others in the same category as well as the histopathologists with <10 years' experience, leading us to tend to agree with Genta *et al*. that other external influences, and in particular non-evidence based elements e.g. a stance of enhanced post-error caution, can lead to variability in histopathologists' choices as well as expanded use of IHC in deriving their diagnoses.[11]

Finally, while the usage of IHC in our centre appears compatible with that of an academic centre, at a rate of IHC performed in 21% of cases with an average of 2.9 stains in each case with IHC carried out, our study also highlights a certain concern regards the possibility of variation in histopathologists' leanings towards usage of IHC. It is probably justified to say that whether rightly or wrongly there is a somewhat tacit advocacy and perception of IHC as a "diagnostic clincher" amongst some histopathologists. This position will therefore carry some inherent problems. The deluge of antibodies currently available in the market can drive a fear of error by not being sufficiently exhaustive in utilising the full range of antibodies to work up a case. Coupled with a constant demand for speedy diagnosis, it is understandable that histopathologists may opt to place more reliance on IHC than the laborious detailed examination of the routine H+E stained section to derive the diagnosis. However, this type of practice is not without its downside. Besides encouraging a more blunderbuss approach than approaching the case with more careful deliberation on the histological features, IHC stains are not without cost. The cost factor worsens when multiple antibodies with only slight variation in determinant role are available to the histopathologists who can be easily beguiled to utilise more than what is really necessary. In an era of escalating healthcare cost, it may be timely and prudent for the profession to formulate some guidelines on the judicious use of IHC that will rationally balance diagnostic accuracy and patient safety against cost to avoid excesses or under-utility.

Authors' contribution: PLC designed the study, analysed the results and wrote the manuscript. YTC collected the data and analysed the results. LML designed study and analysed results.

Conflict of interest: The authors declare no conflict of interest.

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