Dermatofibrosarcoma protuberans: dealing with slow Mohs procedures employing formalin-fixed, paraffin wax-embedded tissue in a busy diagnostic laboratory

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ABSTRACT

Dermatofibrosarcoma protuberans (DFSP) is a relatively uncommon tumour that arises in the dermis and underlying soft tissue. Surgical removal is the preferred treatment, with relatively wide clearance margins of 3 cm or more. Slow Mohs procedures are often employed successfully to treat patients with such tumours. Slow Mohs procedures offer the benefit of improved cure rates and maximal tissue conservation. However, dealing with such tissue successfully presents the laboratory with a host of technical problems. This report advocates a set protocol to follow for slow Mohs, based on the experience acquired from dealing with 37 cases of DFSP over a 12-year period. The report establishes the benefits of slow Mohs paraffin wax-embedded tissue over frozen sections in terms of improved morphology, tissue preservation and immunocytochemical labelling with anti-CD34.

KEY WORDS: Dermatofibrosarcoma protuberans.
Mohs surgery.
Immunohistochemistry.

Introduction

Dermatofibrosarcoma protuberans (DFSP) is a rare sarcoma which is believed to be of fibroblastic or myofibroblastic derivation. It is believed to have been first described as a progressive and recurring dermatofibroma by Darier and Ferrand in 1924, and Hoffman is generally recognised for officially coining the term dermatofibrosarcoma protuberans in 1925.[1] The tumour is of an intermediate- to low-grade type. Although metastasis is rare, it invades locally and has a high recurrence rate. The tumour is commonly found within the dermis but it can invade deep into the subcutis, infiltrating the fatty tissue mainly via the fibrous septa between the adipocytes.

Conventionally, surgical removal represents the treatment of choice, ensuring margins of 3 cm or more of clinically uninvolved skin and underlying fascia. These tumours can expand to relatively large size and, if arising on the head and neck, can result in disfiguring and problematic reconstruction following removal.

Mohs micrographic surgery is widely employed for the removal of more conventional tumours (e.g., basal cell carcinoma) because of established improved cure rates over conventional surgical removal, maximal conservation of normal uninvolved tissue, and often an improved overall cosmetic result. However, the single significant positive indicator for the use of a Mohs procedure is that it enables the examination of the entire surgical margin, a facility not afforded by any other surgical procedure currently available. More recently, its popularity as a treatment for DFSP has been championed.

Frederic Mohs employed his Mohs technique on formalin-fixed tissue to evaluate DFSPs as early as 1978.[2] Thus, the introduction of the slow Mohs concept was introduced. Although the indicators from this report show a promising application for Mohs procedures in the treatment of DFSP, case numbers are relatively low. Indeed, there are relatively few substantive large studies in the literature reporting on the use of slow Mohs in the treatment of DFSP. Of more significant interest from the laboratory perspective is the observation that there are no significant guidelines on how the laboratory should deal with slow Mohs procedures for DFSP.

The present study aims to outline the optimal methods for dealing with such tissue effectively in the laboratory, based on experience obtained over a decade when dealing with a wide variety of DFSP cases. The fundamental question that needs to be answered is why use slow Mohs procedures in preference to conventional frozen section Mohs? The reason is principally that cryostat sections often provided suboptimal preservation of fat, and sections are often damaged and tissue in the fatty deep dermal areas of the tumour is often missing. This can make determination of clearance of the tumour margins difficult. In addition, the morphological features in cryostat sections are not as clearly defined as those seen in formalin-fixed, paraffin wax-embedded tissue sections.[3]

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Dermatofibrosarcoma protuberans can undergo fibromatous change and in such cases good morphological detail of tumour cells is invaluable.[4,5] Often, the problem of producing an intact, full-face section of tissue using a cryostat is improved by increasing the thickness of the section. Normally, tissue section thickness for cryostat work would be 5–10 μm. In many instances, when dealing with fatty tissue from DFSP cases, tissue sections may need to be cut at 15–20 μm in order to ensure that an intact, representative section is obtained. Despite this, an adequate section may still not be produced. At the very least, the morphology resulting from these thicker sections is significantly compromised due to the inherent problems associated with light transmission through a thicker tissue section. Therefore, a slow Mohs approach to dealing with DFSP has clear advantages. Slow Mohs procedures also facilitate the easy production of multiple sections for additional immunocytochemical tests (e.g., anti-CD34). Characteristically, DFSPs show little immunoreactivity; however, in the majority of cases they are positive for CD34, and this can be employed to identify tumour cells in deep margin areas.[6]

Materials and methods

Surgical data

All 37 cases of DFSP removed using slow Mohs procedures were taken from the files of St. John's Institute of Dermatopathology (received between January 1999 and March 2012). All cases were diagnosed initially on conventional haematoxylin and eosin (H&E) preparations and in some cases with the additional use of anti-CD34 immunocytochemical labelling.

The majority of the lesions removed by the Mohs surgical teams involved a surgical debulk of the visibly discernable and palpable tumour area, followed by the removal of the surrounding tissue rim/layer (5–10 mm thick). The surrounding tissue layer was then divided or sectioned into multiple tissue pieces and mapped using the conventional surgical Mohs map (anatomical sheets, Fig. 1). The Davidson Dye System and Delasco Tissue Stain are popular choices in many laboratories as they provide a variety of dye options to colour specimen margins (Fig. 2). While choice of dye is a personal preference, it is important that the dye be applied sparingly as 'bleeding' from one area to another may lead to confusion and an inability to differentiate how the specimen should be correlated to the surgical site.

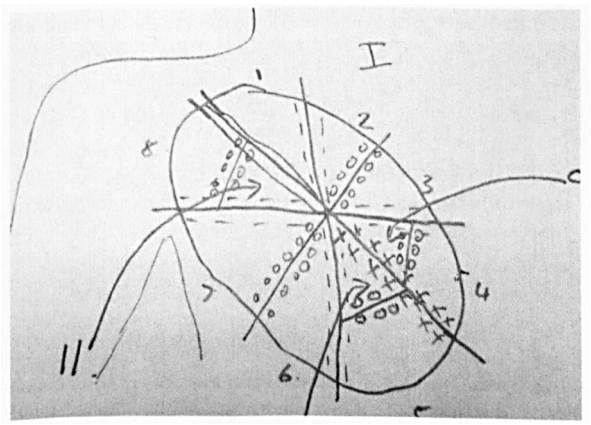

Fig. 1. Mohs surgical map identifying tumour removal and resulting macroscopic tissue dissection.

Fig. 2. Davidson's marking dyes employed to ink the resection margins of the excised tissue.

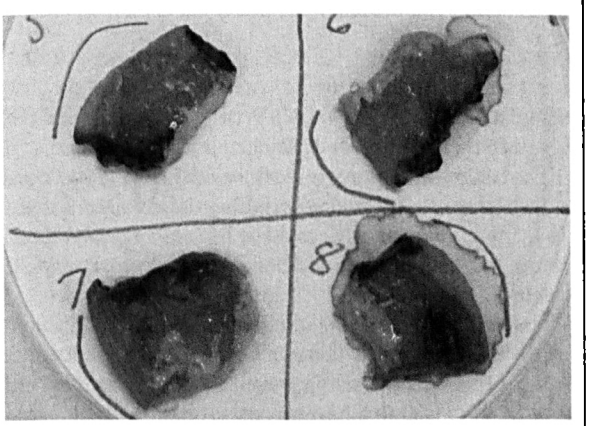

Fig. 3. Tissue pieces inked on the margins in accordance with the Mohs map in Figure 2.
See these images in colour at www.bjbs-online.org

Communication with the laboratory regarding the expected delivery time for the tissue was received in the majority of cases; however, no tissue arrived in the laboratory before 2.00 pm in any of the cases. The number of tissue pieces mapped and sent for processing by the laboratory varied considerably, with anything from three to 23 pieces being received from the first round of tissue removal. All tissue slices were then placed in Petri dishes on top of white filter paper and numbered in accordance with the Mohs map (Fig. 3). Petri dish lids were taped securely and the dishes transferred to the main dermatopathology laboratory.

Tissue fixation and processing was performed to enable visualisation of horizontal examination of the tissue pieces the following day. The evaluation of tumour margin clearance was performed by the Mohs surgical team with support from the resident dermatopathologists. The process was repeated on a daily basis until tumour-free margins were obtained.

Laboratory procedures

All DFSP cases examined were performed on scheduled Mohs clinic days. Surgical teams were involved in

Table 1. Advantages and disadvantages of Mohs procedures for the assessment of DFSP: paraffin wax-processed slow Mohs versus conventional frozen sections.

Method	Fat preservation	Embedding	Morphology	Speed (turnaround time for sections)	Reproducibility/ accuracy and complexity	CD34 labelling	Cost
Frozen	Problematic as thicker sections often required. Tears and holes often occur	Fast and efficient	Generally not ideal, with greater restrictions with thicker sections	Much faster than paraffin (5–10 minutes)	Less reliable, evidence of increased risk of false negatives. Less-complex technical procedures	Owing to issues of tissue preservation, CD34 is less reliable on frozen sections	Much cheaper (reduced labour cost as contact times are reduced)
Paraffin	Generally more reliable and consistent, with good preservation	More complex as a result of tissue curling and shrinking due to processing	Much improved over frozen method, with much thinner sections produced	Much slower than frozen method (18–20 hours with slow Mohs)	More consistent and more accurate than frozen method, but increased complexity of technical procedures	Tissue preservation improved, with more robust tissue sections and improved end results	Far more time-consuming, with increased labour costs

conventional frozen section Mohs cases as well as the DFSP slow Mohs cases on such days.

The laboratory was informed the week before of the Mohs schedules for the given day, including the slow Mohs cases. As many as 10–12 patients could be booked for conventional frozen section Mohs, in addition to any given slow Mohs case(s).

Tissues were delivered to the main laboratory on the floor below the Mohs clinic. It was inked in the normal manner using the Davidson marking system and then placed in Petri dishes for transfer to the laboratory, together with the Mohs anatomical map. All the tissue pieces, including the debulk sample, were labelled on the Petri dish to prevent any confusion.

All cases for slow Mohs procedures were recommended for delivery to the main dermatopathology laboratory no later than 2.30 pm. On arrival, cases were entered on the pathology reporting system (Pathnet). The tissue was then examined by a senior biomedical scientist in conjunction with a member of the surgical team. Discussion would involve the appropriate size of the respective pieces provided, most significantly the depth of each tissue piece.

Ideally, all pieces were evaluated to ensure they could be placed in standard histopathology tissue processing cassettes. The surgical team member would also indicate which samples, if any, included epidermis and which were from a deep margin. A single tissue cassette would then be prepared for each piece of tissue and would be marked as a slow Mohs case. This would normally be indicated by use of a blue cassette, which would stand out among the standard white cassettes normally used for routine surgical cases (Fig. 4).

The tissues would be lifted directly from the Petri dish and placed inside the tissue cassette in exactly the same orientation to ensure that the lower surface would be embedded as the cutting surface of the paraffin block produced after processing. If the pieces were small they would be wrapped in tissue wrap paper and an ink dot placed on the paper to indicate which surface represented the posterior surface. The debulk sample would be sliced into 3-mm slices vertically and placed in one cassette. Records were kept of each cassette on the tissue cut-up log.

Cassettes were then transferred to 10% neutral buffered formalin (NBF) pots, which had be placed in a small 60°C fan oven for 30 min prior to receipt of the DFSP case. Two cassettes were placed in each NBF pot and the lids securely fastened. All pots were then returned to the 60°C oven for 2 h (this is a critical step and fixation times may vary according to the dimensions of the tissue block; however, as an average, 2 h was found to be appropriate for all the cases studied), after which they were removed and placed in a fume hood where the lids were unscrewed and the cassettes transferred to the enclosed tissue processing machine (Leica TP10/50).

The schedule involved a total of 15 hours' processing, and included dehydration in alcohol (7 h), clearing in xylene (3 h) and infiltration with paraffin wax at 60°C (5 h). The processing schedule utilised both vacuum and pressure to maximise penetration of the solvents and wax.

The following morning the tissue cassettes were removed from the processing machine and embedded using a Tissue-Tek tissue embedding centre (Sakura). Tissue samples from each cassette were placed in appropriately sized moulds and orientated according to the Mohs embedding requirement.

Fig. 4. Tissue pieces transferred directly to prelabelled Tissue-Tek processing cassettes.
See this image in colour at www.bjbs-online.org

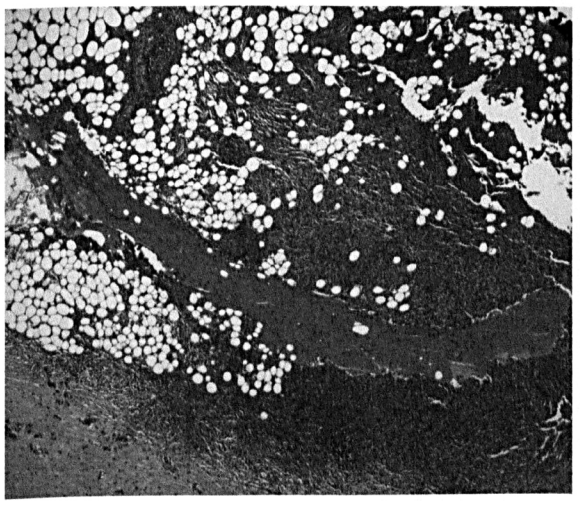

Fig. 5. Haematoxylin and eosin (H&E) staining showing DFSP tumour cells invading subcutaneous fat (original magnification x40)

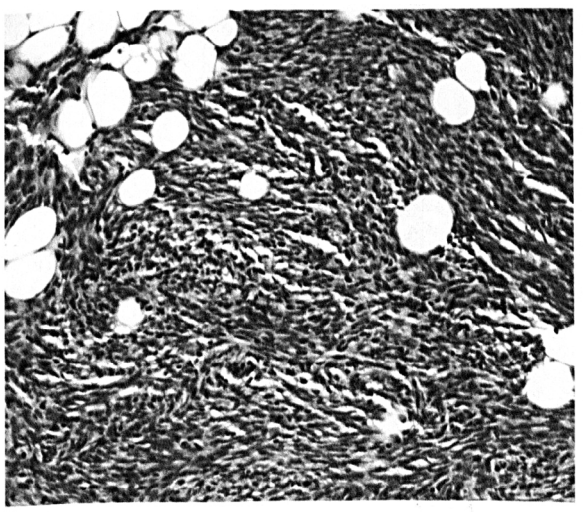

Fig. 6. Tumour cells stained with H&E (original magnification x100).
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All tissues would have undergone some degree of shrinkage during processing, which causes the tissue edges to roll upwards. In order to ensure that the full face of the tissue block was available for section cutting, heated forceps and embedding flat irons were used to press the tissue flat on the base of the mould while the surrounding wax was allowed partially to solidify. The processing cassette was then place on the mould, additional paraffin wax was added, and each block was transferred to the cold plate to solidify completely. Debulk samples were embedded on the cut surface and sectioned vertically, while all the other tissues were embedded horizontally on the outer edge.

Section cutting was performed on a rotary microtome (Leica 2125). Prior to sectioning, in order to conserve tissue, the angle of the blade was checked to ensure that the paraffin block was perpendicular to the knife edge. Tissue blocks were trimmed gently, then three sections (4 μm thick) were cut at 50 μm intervals through the paraffin block, providing three levels for evaluation from each block. Sections were floated out on a water bath (40°C) to enable folds and creases to be removed before being picked up on charged slides and placed in a 60°C oven for 15 min to dry.

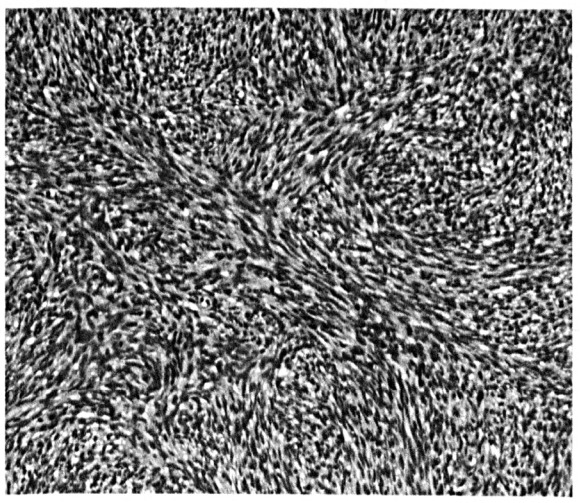

Fig. 7. Tumour cells showing the classical storiform pattern (original magnification x200).

Sections were stained with H&E (Harris's haematoxylin: 7 min, eosin: 3 min; Figs. 5–7). All sections were stained on a staining machine (Leica autostainer XL) and mounted using the CV 5000 coverslipper (Leica). All sections were checked by a senior biomedical scientist to ensure that a full face had been cut and all the epidermis was present, and that the inked margins were visible. If this was not the case, deeper sections were requested.

Slides were then submitted to the dermatopathologist, often accompanied by a member of the surgical team, to assess the sections microscopically to determine if the patient was clear of tumour or if a second round of tissue removal would need to be performed later that day.

In the majority of cases a second or even a third/fourth round of tissue removal was required. In some problem cases the surgical team would request the use of anti-CD34 staining when delivering the tissue to the laboratory. This was performed at the same time as H&E staining. An extra two slides were cut from each block. One would be stored as a spare and the other stained with anti-CD34 (Leica NCL-CD34; Figs. 8 and 9) using an immunostaining machine (Roche BenchMark XT) and detection kit (Roche Ultra). The staining cycle lasted 90 minutes and immunostained slides were produced within 20–30 minutes of the H&E-stained sections being completed.

Results

Clinical details

From 1997 to 2011, 37 cases (20 male [54%], 17 female [46%]) of DFSP were assessed for this study using the slow Mohs procedure. Anatomically. DFSP lesions were removed from the abdominal area, back, arm, leg, head and chest areas. The most common sites were the chest and abdomen. The majority of these tumours (26 [70%]) were primary lesions. The remaining cases were recurrent tumours referred for slow Mohs following incomplete primary excision over a number of years. No patients in this study had metastatic disease.

Laboratory assessments

Following the above procedure, adequate processing of all

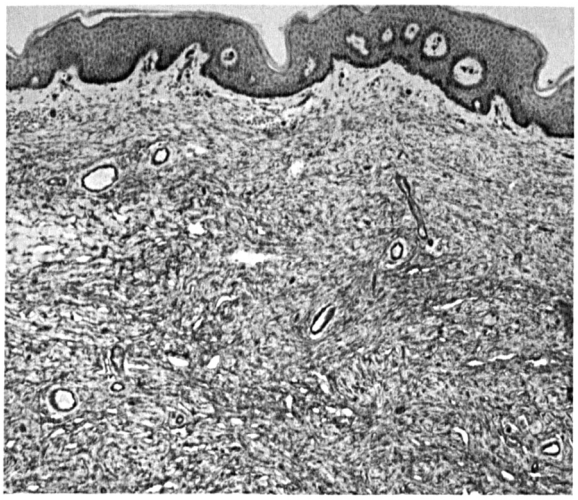

Fig. 8. CD34 labelling of DFSP tumour spindle cells in a debulked sample (original magnification x100).

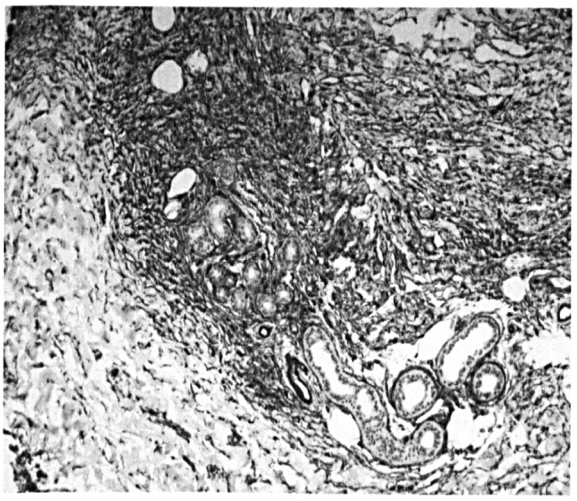

Fig. 9. CD34 labelling of DFSP spindle cells close to a lateral margin (original magnification x100).

but two cases of DFSP assessed in this study was achieved. Adequate processing and staining involved the production of well-stained sections showing good morphological detail and without apparent tearing of the fat. The two cases affected involved large, recurrent tumours from which the tissue blocks were excessively fatty and were too thickly sliced by the surgical teams to enable an appropriate fit into the tissue processing cassette. As a result, some blocks were not adequately fixed and processed. In all cases, this only affected a few blocks and did not pose difficulty with overall interpretation of the lesions removed.

Additional CD34 staining was employed in five cases of the 37 examined. This was useful when tissue margins demonstrated an abundant inflammatory infiltrate, but it was less useful in cases which exhibited fibrosarcomatous change.

Discussion

This study attempts to recommend procedures for the laboratory to follow to enable adequate preparation of slow Mohs cases for DFSP assessment. A review of the literature shows that there is very little information available giving clear indications of what procedures the laboratory should adopt to deal with such cases. More significantly, no comparative studies have been undertaken to evaluate different approaches to this issue.

The significant rationale is that paraffin wax-processed tissue offers the benefit of improved morphology over frozen sections. The DFSP often invades deep into the dermis and infiltrates the subcutis. Tumour cells characteristically are spindle shaped and are described as appearing in a storiform pattern.[4,5] If these cells are present deep in the fat, often between the fibrous septa in and surrounding the fat cells, they can be difficult to identify and distinguish from normal spindle cells present in the dermis. Scar tissue or granulation tissue may also be present in re-excision cases, and spindle cells can also be seen in such cases, particularly in scar tissue. This provides another source of potential confusion.

The use of frozen sections does not allow morphological details of the cells to be defined clearly. In addition, the

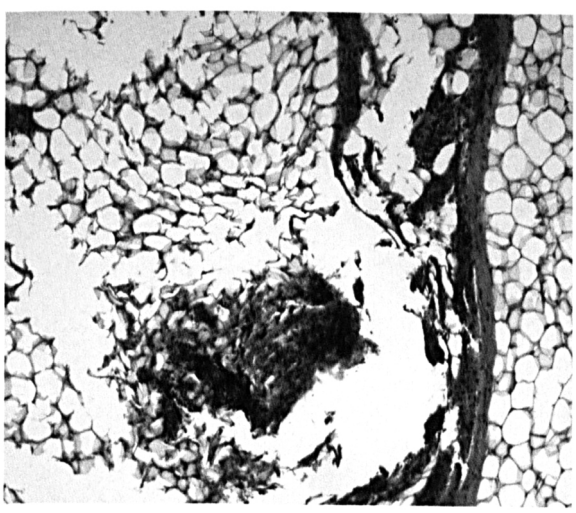

Fig. 10. Inadequate processing of fatty tissue resulting in tearing and poor morphological preservation (original magnification x100).
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preservation of fat represents a problem and often results in inadequate representation of the tissue block due to tearing (Fig. 10). Frozen sections of fatty tissue are also far more likely to detach from slides, even if charged or coated slides are used.

The clear benefit of paraffin wax-processed tissue is that archival records of the material can be stored and reassessed, and immunocytochemical labelling can be employed. However, it should be remembered that CD34 can complicate the assessment of DFSP, particularly in cases showing fibrosarcomatous change. CD34 also labels a naturally occurring dermal spindle cell population, which, to the untrained eye, can be confused with a tumour deposit.[7,8] CD34 is used routinely to demonstrate blood vessels and for the assessment of endothelial cell tumours (e.g., Kaposi's sarcoma, angiosarcoma).[8-10]

Frozen sections of DFSP offer one clear advantage: speed of tissue preparation. In certain circumstances, usually defined by patient management issues, a conventional frozen section approach may be more appropriate. In such cases, the final layer of frozen tissue is often sent for formalin fixation and paraffin wax-processing, and is used to confirm

tumour clearance.[11] However, there have been cases where tumour cells have been found in paraffin wax-processed tissue which appeared to be clear of tumour on frozen section.[12] This supports the notion that formalin-fixed tissue preparation is the more accurate method.

Preservation of fat is not always guaranteed following formalin fixation and paraffin processing. In the authors' experience, the defining factors are tissue sample size, particularly the depth of the tissue in each cassette, the amount of fat present, and the amount of time the tissue is exposed to alcohol dehydration. Even several hours in alcohol may be insufficient to ensure complete and adequate processing in all cases.

In the modern health service, the issue of cost is ever-present, and paraffin wax processing will result in prolonged patient treatment, which can stretch over a number of days. In some cases, tumour removal may be extensive, resulting in the patient being admitted for overnight stay in hospital. Some patients may also experience complications with wound healing, in which case large, open wounds would not be desirable or indeed comfortable for the patient (Table 1, see page 58).

In summary, this study indicates that there are four fundamental areas to consider with slow Mohs laboratory procedures:

1 good communication between the surgical teams and the laboratory staff is needed in planning the slow Mohs procedures
2 understanding of expected turnaround times for delivering histological paraffin wax-embedded sections for evaluation, and an awareness of the fundamental steps that need to be established (e.g., rapid fixation and adequate alcohol processing steps) to ensure these turnaround times are met
3 comprehensive appreciation of how to evaluate tissue samples to determine how adequate they are for rapid fixation and processing, and to be able to convey this information adequately to the surgical teams
4 understanding of the value and use of rapid anti-CD34 staining in difficult cases, which should include the ability to troubleshoot procedures quickly and efficiently to enable corrective action to be taken, if required. □

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