Store-and-Forward Diagnostic Telepathology of Small Biopsies by E-Mail Attachment: A Feasibility Pilot Study with a View for Future Application in Thailand Diagnostic Pathology Services

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ABSTRACT

Diagnostic telepathology by electronic mail (e-mail) attachment is relatively simple and incurs minimal cost. We assessed its accuracy and practical aspects in routine diagnostic pathology. Using 100 small biopsy specimens, a total of 1488 images were digitized by one pathologist and sent as e-mail attachments from Nara Medical University, Japan, to a pathologist at Rajavithi Hospital, Thailand. His diagnoses were compared with his conventional light microscopy interpretation at a later date. The average total turnaround time spent on each case was 215 minutes, far less than the several days required by conventional post. There were two clinically significant errors. One was a diagnostically difficult case of colonic dysplasia, which was called carcinoma with telepathology. The other was a signet ring cell carcinoma of the stomach which was undetected with telepathology. Microscopy objective magnification and digital image quality may have played a role in impairing interpretation in both cases. Store-and-forward telepathology provides acceptable efficacy, a comparatively faster turnaround time than post and could be applied in routine work within Thai pathology services.

INTRODUCTION

In Thailand, there are approximately 250 pathologists serving a population of 60 million. Most can be found in cities, universities, or large hospitals. Unfortunately, more than 90% of the general public hospitals have no pathologist on site. In these hospitals, surgical specimens are sent in formalin to the nearest pathology center. This takes at least 2 days for small biopsies, longer for larger specimens, with the pathology report being returned by facsimile or courier mail. Compared with an average minimum turnaround time of less than a day for small biopsy specimens in a large hospital, it is evident that the patient in a rural

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community is at a significant disadvantage, particularly when management decisions are
dependent on pathology.

Three factors have limited or inhibited the application of telepathology in Thailand. The
first is lack of adequate funding. It was only since the proliferation of home consumer digi-
tal cameras that the prices of digital imaging hardware have fallen enough to be affordable
for an average laboratory. The second factor is the inadequacy of the advanced telecommuni-
cations infrastructure for multimedia communica
tion. Thai Internet services suffer from con-
gestion; even basic services such as e-mail and
Web browsing are unreliable. The third factor
is lack of awareness and education. Thai
pathologists receive no guidance or training in
digital microscopy, and have only basic com-
puter skills.

Telepathology in Thailand has been a spor-
adic activity. One of the earliest recorded ex-
periments in this area occurred in 1997. It in-
volved assessment of images sent through the
Internet via e-mail attachment and down-
loaded using a V.90 modem. In 1998, one of
the authors (K.T.) investigated the viability of
digital images to convey an accurate repre-
sentation of the glass slide. One-hundred-
and-two images from 10 histological and 4
cytological breast cases were digitized in
Germany and sent on CD-ROM to be assessed
in Thailand. The reliability of digital images
was comparable with glass slide assessment,
provided the selection of images was reliable
and the method of digitisation of sufficient
quality.

The largest government-funded exercise oc-
curred between 1998 and 2000, and involved
eight pathologists in Bangkok (Institute of
Pathology and Rajavithi Hospital), and the
provinces of Nakhon Ratchasima in northeas-
tern Thailand (Maharat Nakhon Ratchasima
Hospital) and Nakhon Srithammaraj in south-
eastern Thailand (Maharat Nakhon Srithammaraj
Hospital). It used store-and-forward tech-
niques and e-mail attachment as the conduit.
The Thai government is currently assessing this
work to determine future action. It is clear that
improvements in telecommunications infra-
structure in Thailand will not be motivated by
telepathology alone. Moreover, heavy expen-
diture on digital imaging hardware is unreal-
istic for many laboratories.

We experimented with robotic telepathology
in 2000 using a V.90 modem link between a pri-
vate laboratory in Bangkok (Hi-Tech labora-
tory), Rajavithi hospital and Maharat Nakhon
Ratchasima Hospital. There was >90% concur-
dance between telepathology and light micro-
scopy diagnosis. However the time required
to view each slide was unacceptable for many
users (average 20 minutes).

This study examines the feasibility of using
low-cost digital imaging, mid-range computing
equipment, nonspecialized graphics and e-mail
software, and the Internet for diagnostic
telepathology. Such hardware and software is
now available to most general hospitals. We
assessed diagnostic accuracy compared with
light microscopy, image quality, and total time.
Several studies using store-and-forward
telepathology have commented on the limi-
tation of image sampling. Consequently, we
chose small biopsies to reduce the potential
amount of digital data. This allowed us to digi-
tize all of the tissue area, and thereby reduc-
ning the possibility of misdiagnosis from inade-
quate image sampling.

METHODS

The study was undertaken between Nara
Medical University (Nara, Japan) and Rajavithi
Hospital (Bangkok, Thailand) while one of the
authors (J.S.) was on study leave in Japan.

Thirty-nine sequentially selected small biop-
sies were retrieved from the archives of the Sur-
gical Pathology Unit of Nara Medical Univer-
sity, Japan. Only Hematoxylin & Eosin-stained
sections were used. Each biopsy contained
multiple pieces of tissue, and each piece of tis-
sue was considered separately for the study.

The pieces were randomized, and were con-
sidered as separate cases for this study. They
will be referred to as such in the results. They
were numbered from 1 to 100. The receiving
pathologist was told each case was independ-
ent. Their dimensions and surface areas were
measured and calculated. The entire tissue area
of each piece was digitized using combinations
of 2×, 4×, and 10× objective magnifications
FIG. 1. Number of images required to digitize each case completely and the objective magnifications used to achieve this. Each image was $1600 \times 1200$ pixels in dimension and 24-bit color. The pathologist capturing the images (J.S.) used her clinical judgement to determine the best combination of magnifications to use.
(Polaroid DMC le digital microscope camera; Olympus BH2 microscope with Plan fluor objectives; Macintosh OS9, 256M RAM, 4G hard drive, 17-inch EIZO monitor 832 × 624 pixels @ 24-bit color, Photoshop 5.5). The number of images required to sample the tissue and magnifications were determined by the pathologist (J.S.). Variation among cases can be attributed to the variable content of biopsies and the decisions made by the pathologist on how best to digitize the case. Each individual image was 1600 × 1200 pixels × 24-bit color, and was stored using moderate JPEG compression. The number of images per piece as well as the file size of each image and the time taken to select and digitize the images were recorded.

All images were transmitted as e-mail attachment along with a text file of brief clinical data from Nara, Japan, to a pathologist in Bangkok, Thailand. Connection to the Internet was via shared single ISDN line (maximum bandwidth 64-kilobits/sec). The receiving pathologist downloaded e-mail via V.90 modem (theoretical maximum bandwidth 56 kilobits/sec) and viewed the images on a Windows PC with graphics software (Pentium III 733 MHz, Win98, 128M RAM, 15G hard drive, 17-inch monitor 1280 × 1024 pixels @ 24-bit color; Photoshop 5.5). He replied via e-mail with a diagnosis, time received, time spent assessing images, and a comment on image quality. The total time from image acquisition to receipt of opinion was noted. The cases were then viewed with conventional light microscopy several weeks later, where the receiving pathologist was instructed to render a separate diagnosis on each piece of tissue, rather than the entire slide as a whole.

When there was discordance between telepathology and light microscopy diagnosis, both images and glass slides were reassessed to determine the reason for the error.

**RESULTS**

The surface area per case (i.e., per piece of tissue) ranged from 0.5 to 29.2 mm$^2$ with mean of 4.5 mm$^2$. A total of 1488 images was digitized, varying between 2 and 35 per case with an average of 14.9. Figure 1 shows the number of images captured per case and the objective magnifications used. Compressed image sizes (JPEG) varied between 64 and 336 KB (mean, 204.5 KB). Cumulative file size for each case varied between 376 and 7,239 KB (mean, 3043.5 KB).

The range of specimens spanned a typical gamut that a general pathologist may expect to see. Table 1 outlines this in more detail. There

<table>
<thead>
<tr>
<th>Organ</th>
<th>Total (pieces)</th>
<th>Diagnosis (rendered independently on each piece of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric biopsy</td>
<td>58</td>
<td>Gastritis without dysplasia (17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gastritis with dysplasia (38)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenocarcinoma (3)$^a$</td>
</tr>
<tr>
<td>Colonic biopsy</td>
<td>22</td>
<td>Normal (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Colitis (11)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tubular adenoma (3)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dysplasia (5)$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Adenocarcinoma (2)</td>
</tr>
<tr>
<td>Esophageal biopsy</td>
<td>5</td>
<td>Esophagitis (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Squamous cell carcinoma (2)</td>
</tr>
<tr>
<td>Bronchial biopsy</td>
<td>5</td>
<td>Mild chronic inflammation with anthracosis (5)</td>
</tr>
<tr>
<td>Liver, needle biopsy</td>
<td>2</td>
<td>Caseating granulomatous inflammation (2)</td>
</tr>
<tr>
<td>Prostate, needle biopsy</td>
<td>2</td>
<td>Prostatic intraepithelial neoplasia (low grade) (2)</td>
</tr>
<tr>
<td>Lymph node biopsy</td>
<td>2</td>
<td>Reactive hyperplasia (2)</td>
</tr>
<tr>
<td>Rectal biopsy</td>
<td>2</td>
<td>Adenocarcinoma (2)</td>
</tr>
<tr>
<td>Skin biopsy</td>
<td>1</td>
<td>Malignant melanoma (1)</td>
</tr>
<tr>
<td>Endocervical biopsy</td>
<td>1</td>
<td>Endocervical polyp (1)$^a$</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

$^a$Discordance between telepathology and conventional light microscopy.
were four instances where telepathology differed from light microscopy and in only two of these were they clinically significant (see Table 2). Case 10 was called adenocarcinoma with telepathology but only colonic dysplasia with conventional light microscopy. Case 95 was regarded as dysplastic with telepathology but diagnosed with light microscopy as infiltrating signet ring cell carcinoma of the stomach. Figures 2 and 3 show a representative image from each of these cases.

The receiving pathologist rated the quality of the images as good or fair for 4× and 10× objective and poor for the 2× objective. It took between 4 and 44 min (mean, 21.4 min) to capture all the images required per case, although this was largely dependent on the experience of the operator. The times of the first 10 pieces ranged from 2 to 3.5 min per picture and improved to 1.2 to 1.8 min in the last 10 cases. The receiving pathologist, spent between 1 and 41 minutes per case to form a diagnosis (mean, 7 minutes). The total time spent (including time to acquire images and Internet e-mail transmission delays) varied between 24 and 1074 minutes per case (mean, 215 minutes).

**DISCUSSION**

The classification of telepathology systems is likely to change with recent advances in complete slide digitization, and indeed Weinstein’s classification reflects this evolution. Nonetheless, a simplified approach is to regard telepathology in terms of robotic (dynamic) systems or store-and-forward (static) systems. The essential difference between the two is the ability to view images ‘live’ as they are seen under the microscope, rather than capturing them and sending them (store-and-forward). Dynamic systems may exist with or without microscope robotics, but remain expensive both in terms of hardware and required bandwidth for satisfactory operation. Those laboratories in Europe, the United States, and Asia that can afford to use such systems have demonstrated high concordance rates with conventional glass slide diagnosis.

In contrast, store-and-forward telepathology may be employed by anyone with digital microscope camera and an Internet connection. This study was initiated by pathologists with no external technical assistance and little advance training. Even so, the concordance with light microscopy (96%) is on par with past studies. Accuracy of store-and-forward telepathology has varied between 85% and 100%. Many now realize that accuracy may be influenced by the person capturing the images.

In this study, each piece of tissue from a biopsy case was considered independently. This allowed the localization of interpretive errors. Typically, there is sufficient variation between pieces of tissue within a single biopsy, e.g., a gastric biopsy, to avoid repetition and to warrant considering each piece separately. The case breakdown (Table 1) supports this.

### Table 2. Cases in Which There Was Discordance Between Telepathology and Light Microscopy

<table>
<thead>
<tr>
<th>Case</th>
<th>Organ</th>
<th>Telepathology diagnosis</th>
<th>Light microscopy diagnosis</th>
<th>Surface area (mm²)</th>
<th>Number of images used</th>
<th>File size (KB)</th>
<th>Possible reason for discordance</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Colon</td>
<td>Adenocarcinoma</td>
<td>Dysplasia</td>
<td>4.18</td>
<td>15</td>
<td>2,989</td>
<td>Diagnostically difficult case; poor image quality</td>
</tr>
<tr>
<td>18</td>
<td>Endocervix</td>
<td>Cervicitis</td>
<td>Endocervical polyp</td>
<td>29.24</td>
<td>29</td>
<td>7,239</td>
<td>Unable to demonstrate low power architecture adequately with multiple images</td>
</tr>
<tr>
<td>19</td>
<td>Colon</td>
<td>Colitis</td>
<td>Tubular adenoma</td>
<td>21.15</td>
<td>24</td>
<td>4,958</td>
<td>Similar reason to case 18</td>
</tr>
<tr>
<td>95</td>
<td>Stomach</td>
<td>Dysplasia</td>
<td>Signet ring cell carcinoma</td>
<td>2.73</td>
<td>13</td>
<td>2,621</td>
<td>Suboptimal image quality; poor contrast and weak color</td>
</tr>
</tbody>
</table>
Cases 18 (endocervical polyp) and 19 (tubular adenoma) were two of the biggest specimens in our series (29.2- and 21.1-mm² surface area, respectively). They required 29 and 24 images, respectively, to digitize all of the tissue. Both of these diagnoses require appreciation of the entire architecture at low power, but the receiving pathologist had to contend with disjointed images. In such a situation, having an image of the entire piece of tissue would be important. This was not possible given the camera field of view and microscope optics.

Case 10 (Fig. 2A) is a typical diagnostic dilemma—dysplasia versus carcinoma—usually difficult even with conventional light microscopy. An improvement in image quality may have assisted assessment (Fig. 2B). We believe that in diagnostically difficult circumstances, quality of the digital image quality can influence outcome. This was evident in the one false-negative case (case 95) in which difficulties in identifying a focal collection of malignant signet ring cells were compounded by inadequacies in the digital image (Fig. 3). The quality of these images is typical of those used in the study. In print, these images may fail to convey the impression given when viewed on a computer monitor but, to a critical eye, the image was suboptimal. The colors were weak and the contrast poor. The degree of JPEG compression produced characteristic ‘8 × 8 pixel blockiness’ artefact, evident when the image was digitally magnified to 200% original (Fig. 3B). JPEG compression on its own was not to blame, and, indeed, its use has gained acceptance in telepathology.\cite{18} However, image-processing algorithms to the original JPEG image to improve the quality, color, and contrast without introducing spurious data may have altered the pathologist’s impression. This is demonstrated in Fig. 3, C and D, which arguably shows the diagnostic information more clearly.

Although simple to implement, such skills are beyond the average diagnostic pathologist. They do not require any extra hardware or expense, but rather increased knowledge and digital image experience on the part of the pathologist. It is also of note that none of these cases used objective magnifications of greater than 10×, probably because the transmitting pathologist felt it unnecessary given that she could make the diagnosis at lower magnifications. In hindsight, we realize that using a higher magnification may offset any digital artefacts, and make it easier for the receiving pathologist to interpret fine detail. Finally, one must also consider the importance of computer monitor calibration at the receiving end.

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FIG. 2. (A and B) A false-positive case. This one of the 15 images transmitted to represent case 10. The crush artefact and large dysplastic cells resulted in an overdiagnosis of malignancy when viewed on a monitor. Image A shows the original image used in the study. Image B shows the effect of image processing to accentuate diagnostic features. Interpretation may be assisted through careful image processing, however distinguishing colonic dysplasia from overt malignancy is a diagnostically difficult area. Please note: In order to appreciate the true difference between these images, this figure may be downloaded in full color from http://telepathologycity.com/publications.htm (H&E, 10× objective).
Ongoing issues in telepathology have been discussed extensively by previous authors and are the subject of numerous national and international committees.\textsuperscript{1,5} Cost is a primary concern for the individual laboratory. Robotic systems are expensive, but store-and-forward systems are now affordable. Diagnostic accuracy has been shown to improve with use,\textsuperscript{6,11} but robotic telepathology is recognized as being better than store-and-forward in this area,\textsuperscript{19} primarily due to inadequate or inappropriate sampling of images. However, with the development of total slide digitization systems, store-and-forward systems are likely to change.

Reliability of the service is also an important consideration. Internet mail services fluctuate in Thailand for a variety of reasons. This may be minimized by employing multiple routes of transmission such as public Web-based e-mail services offered at Hotmail, Yahoo, and TelepathologyCity, virtual hard drives, or Web-based image sharing services.\textsuperscript{20,21} Security and medico-legal issues are also of concern. The Internet is open and insecure. Encryption strate-

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{(A and B) A false-negative case. This is one of the 13 images used to represent case 95 and shows diagnostic signet ring carcinoma cells intermingled with mononuclear inflammatory cells. These went undetected by the receiving pathologist, perhaps due to suboptimal image quality. Image A is the original image used in the study. It demonstrates poor color intensity and contrast. Image B is an enlargement showing the noticeable JPEG ‘8 × 8 blockiness’ artefact, which impairs the appreciation of the diagnostic malignant cells. Images C and D show that with image processing the diagnostic information may be demonstrated more clearly. These images have better color saturation, more accurate color representation, higher contrast, and less JPEG artefact. Please note: In order to appreciate the true difference between these images, this figure may be downloaded in full color from http://telepathologycity.com/publications.htm (H&E, 10× objective).}
\end{figure}
gies and guidelines on personal information to be transmitted can reduce the risk. In many countries, such as Japan, Germany, and Canada, medico-legal responsibility for a case diagnosed using telemedicine has already been considered.

Time remains a significant drawback. Telepathology is undoubtedly slower than conventional light microscopy. While it may be faster than sending glass slides in the post, it occupies more of the pathologists' time, which could be spent dealing with local cases. As this study has shown, skill is required in image acquisition, and some degree of image processing is invariably necessary.

The time spent per case in this study was longer than those of past studies reviewed. The single ISDN line in Nara was shared among other members of the department and the V.90 modem connection was accessed through a commercial Internet service provider. Although this is not practical where time is crucial, such as intraoperative frozen section, it is satisfactory for the routine surgical diagnosis and consultation and still represents a marked improvement compared with current methods. Eventually, conditions and infrastructure will improve in Thailand, resulting in improved times. Nonetheless, even with current circumstances, we have demonstrated the feasibility of using e-mail attachments for diagnostic telepathology of small biopsies. Relatively simple equipment provided satisfactory reliability and rapid turnaround time. It represents a solution to the problem of disproportionate distribution of diagnostic pathology services in Thailand. In the near future, the advances and advantages of this tool will overcome its limitations. Store-and-forward telepathology for small biopsy interpretation is viable and cost effective, but it requires an appreciation of a new set of artefacts and a new set of digital imaging skills.

ACKNOWLEDGMENT

This study was supported by a Nara Medical University scholarship, from September 1, 2000, to November 30, 2000.

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