Cancer Grading by Fourier Transform Infrared Spectroscopy

PAUL G. L. ANDRUS,1 ROBERT D. STRICKLAND1 2

1 Department of Pathology, McMaster University, Hamilton, Ontario L8N 3Z5, Canada
2 Department of Laboratory Medicine, Hamilton Health Sciences Corporation, Hamilton, Canada

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ABSTRACT: Thirty-nine freeze-dried tissue samples from 17 lymphoid tumors (nine malignant non-Hodgkin’s lymphomas) were studied by Fourier transform infrared (FTIR) spectroscopy. The absorbance ratio $A_{1121}/A_{1020}$ increased, along with the emergence of an absorbance pulse at 1121 cm$^{-1}$, with increasing clinicopathological grade of malignant lymphoma. An increasing $A_{1121}/A_{1020}$ ratio from benign to malignant is evident in literature spectra from several different tissues; however, the present study is the first to comment on this effect and to propose it as an index of the cellular RNA/DNA ratio after subtraction of overlapping absorbances, if present, due to collagen or glycogen. Absorbance attributable to collagen increased with lymphoma grade and was greater in benign inflammatory tumors than in low-grade lymphomas. The $A_{1121}/A_{1020}$ trend observed here may form the basis of a universal cancer-grading parameter to assist with cancer treatment decisions and may also be useful in the analysis of cellular growth perturbation induced by drugs or other therapies. Our spectral findings may potentially be applied to cell clusters and discrete areas of tumor tissue sections using the FTIR microscope, allowing correlation with morphology and a high degree of spatial resolution. © 1998 John Wiley & Sons, Inc. Biospectroscopy 4: 37–46, 1998

Keywords: FTIR; cancer prognosis; cancer grade; tissue spectroscopy; lymphoma

INTRODUCTION

The expected clinical grade of many cancers, including malignant lymphoma, has important treatment implications. Aggressive (high-grade) lymphomas are treated with chemotherapy with the possibility of cure. Indolent (low-grade) lymphomas are chronic diseases lasting many years but paradoxically are usually not curable. They are treated only when it is needed for symptom relief. The most important diagnostic distinction with respect to treatment is between relatively aggressive and relatively indolent lymphomas. Cancer (including malignant lymphoma) grading by light microscopy is subjective and imprecise. Flow cytometric and immunohistochemical methods of quantifying cellular nucleic acid content or proliferative activity are relatively labor intensive and operator sensitive to perform. These methods do not probe biomolecular structure but depend on sampling large numbers of cells from which the fraction of cycling cells, or the cell DNA content distribution, can be derived.

Malignant tumors are by nature heterogeneous, and their behavior is best characterized by that of the most aggressive subpopulations of cells within them. Given the ease with which infrared spectroscopy can be performed on small tissue or cell samples (0.01 mg by method of Rigas et al.1) with high precision and minimal preparation and
Materials and Methods

All cases of lymphoid tumors biopsied at McMaster University Medical Centre between December 1994 and April 1996 for which sufficient un-embedded tissue was snap frozen were included in the study. These included nine non-Hodgkin's lymphomas, two cases of Hodgkin's disease, and six reactive inflammatory tumors.

Tissue samples, snap frozen in liquid nitrogen-cooled isopentane at the time of excision, were obtained from −70°C storage and vacuum freeze dried on a liquid nitrogen-cooled multiwelled copper plate. Infrared spectra were obtained from potassium bromide (KBr) discs using a Perkin-Elmer 1600 FTIR spectrophotometer (The Perkin-Elmer Corp., Norwalk, CT). For each test, 3 mg of dry tissue was ground to fine flakes with mortar and pestel. KBr, 150 mg, was then added with further grinding and mixing. The mixture was then pressed in a die at 8 metric tons force for 10 s, creating a 1.3-cm-diameter translucent disc with embedded tissue. Sixteen scans at 4-cm⁻¹ resolution were signal averaged for each test. Depending on tissue availability, cases were tested two or three times each by taking separate pieces of tissue from different locations within the tumor and imbedding in separate KBr discs. Histological sections of the tumors from lymph nodes showed these nodes to be totally effaced by tumor. Spectra were viewed in the absorbance vs. frequency mode in the frequency range of 400–4000 cm⁻¹ (Figs. 1 and 2).

Figure 1. Infrared spectra in the frequency region 400–4000 cm⁻¹ of freeze-dried tissue from five relatively clinically aggressive non-Hodgkin’s lymphoma tumors (cases 1–5).

Figure 2. Infrared spectra in the frequency region 400–4000 cm⁻¹ of freeze-dried tissue from four relatively clinically indolent non-Hodgkin’s lymphoma tumors (cases 6–9).
infrared spectroscopy of lymphoma tissue

Figure 3. Signal-averaged infrared spectra in the frequency region 900–1150 cm\(^{-1}\) of tissue from malignant lymphoma cases 1–3 (aggressive), cases 4 and 5 (intermediate), and cases 6–9 (indolent). Cases 4 and 5 (both intermediate) are T-cell non-Hodgkin’s lymphomas, whereas all other cases (1–3, 6–9) are B-cell non-Hodgkin’s lymphomas.

RESULTS AND DISCUSSION

Absorbance on the 1121-cm\(^{-1}\) shoulder of the symmetric phosphodiester stretching band (SPSB) of nucleic acids was found to rise relative to absorbance on the 1020-cm\(^{-1}\) shoulder (1121/1020 index), with increasing clinicopathological grade of the nine cases of malignant non-Hodgkin’s lymphoma studied here (Fig. 3 and Table I). Reproducibility was good as demonstrated in Figure 4, in which the three tissue sample spectra are superimposed for each of cases 3 and 7. Of particular note is the progressive emergence of a 1121-cm\(^{-1}\) centered absorbance pulse with increasing grade (and increasing 1121/1020 index) as seen in Figure 5. The correlation between the 1121-cm\(^{-1}\) absorbance pulse and 1121/1020 index was also evident for benign inflammatory tumors (Fig. 5 and Table II).

Also noteworthy is the increasing relative erosion of absorbance in the 1050–1080-cm\(^{-1}\) region with increasing grade (Fig. 3). This effect is particularly prominent for cases 4 and 5 (intermediate), which happen to be the only two T-cell lymphomas (the other seven cases are of B-cell origin). A similar erosion effect is evident in spectra of oxidatively damaged DNA from breast cancer cells. In normal inflammation, activated T cells trigger macrophages to become activated and secrete toxic oxygen metabolites. Possibly the greater 1050–1080-cm\(^{-1}\) erosion in cases 4 and 5 indicates a greater degree of oxidative damage to DNA in these T-cell lymphomas; however, on the basis of only two cases, this is highly speculative.

Clinical Features

Cases 1–3 were aggressive histologically and clinically. High-grade lymphomas are paradoxically more treatment responsive. Cases 4 and 5 were of intermediate clinical behavior with constitutional symptoms such as significant weight loss. Case 4 was that of a debilitated 84-year-old man who presented with advanced disease and elected to have palliative care only. Cases 6–8 all have indolent clinical courses of 5–8 years duration and all were treated with at least one course of chemotherapy followed by relapse, which is typical of low-grade lymphoma. Case 9 had an axillary node detected on routine examination with bone marrow involvement and was symptom free 16 months later having never received treatment, suggesting very indolent disease.

The cut points between aggressive, intermediate, and indolent are arbitrary because the cases are compared with each other along the continuum of disease severity. Even cases with the same diagnosis can demonstrate significantly different clinical behavior. For example, case 2 presented with a perforated bowel due to necrotic tumor, necessitating emergent surgery, whereas cases 6 and 8 (also diffuse small cleaved cell) showed no such aggressive behavior.

Vibrational Assignments

The SPSB of nucleic acids centered around 1084 cm\(^{-1}\) is mainly due to absorbance by the phosphodiester bonds of the phosphate/sugar backbone of nucleic acids. In glycogen-poor cells such as colonocytes or lymphocytes, the 1121-cm\(^{-1}\) shoulder of the SPSB is primarily due to absorbance by RNA, whereas the 1020-cm\(^{-1}\) shoulder is due to DNA.
Nine cases of non-Hodgkin's lymphoma are ranked in order of their relative infrared spectral absorbances at 1121 and 1020 cm\(^{-1}\) (1121/1020 index), and this parameter predicts for the presence of high clinicopathological grade features such as high mitotic rate (HMR) or necrosis (N), and complete remission after chemotherapy (CR) or constitutional symptoms (CS) designated as present (+) or absent (−). DLBC, diffuse large B-cell lymphoma; DSCI, diffuse small cleaved cell lymphoma; ALTC, AILD-like T-cell lymphoma; TGLD, T-gamma lymphoproliferative disorder; DSL, diffuse small lymphocytic lymphoma; FMC, follicular mixed cell lymphoma; F/U, follow-up period after initial presentation; DOD, dead of disease.

Considering this evidence, it might be expected that more clinically aggressive neoplasms with more cycling cells and/or fewer apoptotic cells, and therefore greater biosynthetic activity, should demonstrate greater absorbance at 1121 cm\(^{-1}\) (RNA) relative to that at 1020 cm\(^{-1}\) (DNA) when compared with indolent neoplasms. The 1121-cm\(^{-1}\) (RNA) pulse that we observed for malignant lymphomas to have a higher average cellular RNA/DNA ratio when compared with low-grade non-Hodgkin's Lymphomas,\(^{13}\) and cellular RNA/DNA ratio during G1 and S phases has been found to be higher in exponentially growing cells than in noncycling cells.\(^{14}\) Recent evidence also suggests a possible direct molecular link between transcription and the cell cycle.\(^{15}\)

Figure 4. Superimposed individual spectra in the frequency region 900–1150 cm\(^{-1}\) of three tissue samples for each of cases 3 and 7 demonstrating a high degree of intratumor reproducibility.

### Table 1. High-Grade Malignant Features Versus 1121/1020 Index Rank

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis (Stage)</th>
<th>High-Grade Features</th>
<th>1121/1020 Index</th>
<th>Follow-Up (Months)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HMR/N CR/CS Tests</td>
<td>Average</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>DLBC (3A)</td>
<td>+HMR +CR</td>
<td>1.77</td>
<td>20</td>
</tr>
<tr>
<td>2</td>
<td>DSCI (4A)</td>
<td>+N +CR</td>
<td>1.74</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>DLBC (2A)</td>
<td>+HMR/N +CR?</td>
<td>1.68</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>ALTC (3B)</td>
<td>– +CS</td>
<td>1.68</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>TGLD (3B)</td>
<td>– +CS</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>DSCI (3A)</td>
<td>– –</td>
<td>1.51</td>
<td>51</td>
</tr>
<tr>
<td>7</td>
<td>DSL (4A)</td>
<td>– –</td>
<td>1.49</td>
<td>96</td>
</tr>
<tr>
<td>8</td>
<td>DSCI (3A)</td>
<td>– –</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>FMC (4A)</td>
<td>– –</td>
<td>1.36</td>
<td></td>
</tr>
</tbody>
</table>
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...sorbance around 1084 cm⁻¹). Alternative parameters will yield the same ranking of cases. For example, a minimum absorbance common to all cases in Figures 1 and 2 is 903 cm⁻¹, and so an alternative parameter could be \((A_{1121} - A_{903})/(A_{1020} - A_{903})\).

Two studies for which the baseline was not corrected in the region between the phosphodiester stretching bands (1150–1200 cm⁻¹) show evidence that it should not be corrected for the relative absolute absorbance type of analysis used in the present study. In spectra of endometrial cancer, \(A_{1150-1200}\) rises considerably relative to other band peaks at 971, 1400, and 1455 cm⁻¹ for grade 3 cancer cells compared with normal cells (i.e., no effect from collagen). Most, if not all, of this baseline rise in the region 1150–1200 cm⁻¹ can be accounted for by an absolute increase in absorbance of both the symmetric and asymmetric phosphodiester stretching bands, with consequent further merging of their neighboring shoulders. These neighboring shoulders are nearer in the spectrum of RNA than in that of DNA, which would further enhance 1150–1200-cm⁻¹ absorbance attributable to RNA. In our lymphoma spectra (Fig. 6), \(A_{1150-1200}\) does rise relative to \(A_{1084}\) with grade; however, increasing collagen absorbance at 1240 cm⁻¹ would be expected to contribute to this effect as well as increasing RNA.

Relative Absorbance Quantification

The relative absorbances on the 1121- and 1020-cm⁻¹ shoulders of the SPSB were quantified in the present study essentially as the absorbance at 1121 cm⁻¹ divided by the absorbance at 1020 cm⁻¹. This was quantified precisely and reproducibly as \((A_{1084} - A_{1020})/(A_{1084} - A_{1121})\) by using the absorbance at the band peak (1084 cm⁻¹) as the reference. This ratio is the difference in absorbance between the band peak (1084 cm⁻¹) and the 1020-cm⁻¹ shoulder divided by the difference in absorbance between the band peak and the 1121-cm⁻¹ shoulder and is referred to in the present study as the 1121/1020 index. It rises as the absorbance profile of the SPSB moves closer to that of RNA and further from that of DNA (i.e., the 1121/1020 index of RNA is much higher than that of DNA, both having their maximum absorbance around 1084 cm⁻¹). Alternative parameters will yield the same ranking of cases. For example, a minimum absorbance common to all cases in Figures 1 and 2 is 903 cm⁻¹, and so an alternative parameter could be \((A_{1121} - A_{903})/(A_{1020} - A_{903})\).

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Collagen Effects

On reviewing spectra in a recent study of endometrial cancer tissue and cells, we observed changes at 1121 and 1020 cm⁻¹ with endometrial cancer grade that resemble our lymphoma results, in both endometrial cancer tissue and endometrial cancer cells (cells being free of collagen and other stromal components, whereas the normal tissue showed significant collagen absorbance). We also noted that the presence (endometrial tissue) or absence (endometrial cells) of collagen made little difference to the relative changes in 1020- and 1121-cm⁻¹ absorbances going from benign to grade 3 cancer. Contrary to the endometrial cancer results in which collagen content decreased with grade, we noted collagen content to increase with lymphoma grade as shown by a broad-based increase in absorbance around 1084 cm⁻¹). Alternative parameters will yield the same ranking of cases. For example, a minimum absorbance common to all cases in Figures 1 and 2 is 903 cm⁻¹, and so an alternative parameter could be \((A_{1121} - A_{903})/(A_{1020} - A_{903})\).

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Table II. Tumors Comprised Primarily of Reactive Inflammation

<table>
<thead>
<tr>
<th>Case</th>
<th>Diagnosis</th>
<th>Tests</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>Cat scratch with necrotizing granulomas</td>
<td>1.55</td>
<td>1.54</td>
</tr>
<tr>
<td>11</td>
<td>Nodular sclerosing Hodgkin’s disease</td>
<td>1.56</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.48</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Nodular sclerosing Hodgkin’s disease</td>
<td>1.51</td>
<td>1.52</td>
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<tr>
<td></td>
<td></td>
<td>1.52</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Florid reactive with focal abscess formation</td>
<td>1.41</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Granulomas in keeping with cat scratch disease</td>
<td>1.41</td>
<td>1.44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Florid reactive with focal noncaseating granulomas</td>
<td>1.37</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.48</td>
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<tr>
<td>16</td>
<td>Benign hyperplasia</td>
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<td></td>
<td></td>
<td>1.46</td>
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<tr>
<td>17</td>
<td>Reactive hyperplasia</td>
<td>1.30</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.37</td>
<td></td>
</tr>
</tbody>
</table>

Eight cases of tumors comprised primarily of reactive inflammatory cells as designated by the original sign-out diagnoses are ranked in order of their relative infrared spectral absorbances at 1121 and 1020 cm$^{-1}$ (1121/1020 index).

between 1200- and 1700-cm$^{-1}$ (Fig. 6) relative to 1084-cm$^{-1}$ absorbance, in keeping with the range of the major collagen absorbance bands as demonstrated by Jackson et al.$^{17}$ Indeed, the pathology reports for cases 2 and 3 commented on the presence of significant quantities of geographic necrosis and sclerosis, and the $A_{1540}/A_{1084}$ ratio was particularly high for these two cases. If collagen or some other protein absorbance were responsible for the 1020- and 1121-cm$^{-1}$ shifts we observed, then the trend would not be expected to hold for tissue in which collagen decreases with malignancy or grade (e.g., endometrial tissue$^{16}$) and for tissues in which collagen increases with grade (our results).

Increased collagen would be expected to slightly lessen the 1121/1020 index because collagen shows minor absorbances at 1082 and 1031 cm$^{-1}$ but no absorbance peak at 1121 cm$^{-1}$. This is consistent with spectra of tissue from several epithelial malignancies that have shown decreasing collagen going from benign to malignant due to destruction of epithelial basement membranes and stroma by invasive cancer cells. These spectra show an exaggerated rise in 1121-cm$^{-1}$ absorbance relative to 1020-cm$^{-1}$ absorbance when compared with our lymphoma results because, we propose, of the simultaneous effect of an increasing RNA/DNA ratio and decreasing collagen within these malignant epithelial tissues$^{1,16,18,19}$ (methodological differences may be a factor here as well). It follows that the 1121/1020
INFRARED SPECTROSCOPY OF LYMPHOMA TISSUE

The absorbance profile in the region of the phosphodiester stretching bands (900–1350 cm\(^{-1}\)) is primarily due to some combination of DNA and RNA, and this profile moves closer to that of RNA and further from that of DNA with increasing cancer grade.

**Glycogen Effects**

Glycogen absorbance was neither observed nor expected in lymphoma tissue. Examination of spectra of malignant cervical tissue shows minimal glycogen absorbance and an \(A_{1084}/A_{1240}\) ratio consistent with relatively uncontaminated nucleic acid absorbance. Normal cervical tissue, however, shows evidence that the peak absorbance at 1084 cm\(^{-1}\) is significantly elevated by the large glycogen band centered at 1025 cm\(^{-1}\), resulting in a grossly elevated \(A_{1084}/A_{1240}\) ratio.

Both collagen or glycogen overlapping absorbances, if present, could be interactively subtracted to reveal an absorbance profile in the region of the symmetric and asymmetric phosphodiester stretching bands that is somewhere along the continuum between that of DNA and that of RNA, this profile in general and the 1121/1020 index in particular) potentially forming the basis of a universal tumor-grading parameter.

**Inflammatory Tumors**

We also studied eight cases of tumors comprised primarily of reactive inflammatory cells ranging in 1121/1020 index from 1.34 for reactive hyperplasia to 1.54 for cat scratch disease with necrotizing granulomas (Fig. 5 and Table II). Hodgkin’s disease (cases 11 and 12) is an unusual cancer that the malignant Hodgkin’s cells usually comprise only a small minority of the tumor cells, the vast majority being reactive inflammatory cells. As can be seen from Tables I and II, separation of inflammatory from malignant processes is not possible with the 1121/1020 index alone. This is not unexpected because reactive inflammatory lymph nodes are associated with mitotically active germinal centers and activated macrophages (granulomas) and therefore significant biosynthetic activity. Many of these reactive conditions are more clinically active (i.e., larger, faster growing tumors with fever) than an indolent lymphoma. The 1121/1020 index thus may reflect the biosynthetic activity of a lesion whether it is inflammatory or malignant. This is supported by

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**Figure 7.** Signal-averaged infrared spectra in the frequency region 400–4000 cm\(^{-1}\) of tissue from six cases of benign inflammatory tumors (cases 10, 13–17) with an average 1121/1020 index of 1.44, compared with signal-averaged spectra of tissue from four cases of indolent lymphoma (cases 6–9) with an average 1121/1020 index of 1.45. The peak absorbance \(A_{1084}\) is standardized relative to \(A_{903}\).
the observed correlation between the 1121-cm\(^{-1}\) absorbance pulse and 1121/1020 index in both benign and malignant tumors (Fig. 5).

After controlling for the 1121/1020 index, an important difference became evident when comparing reactive inflammatory tumors with lymphomas (Fig. 7). Inflammatory tumor tissue showed increased \(A_{1200-1700}\) compared with low-grade lymphomas. This effect held for cases 16 and 17 (simple reactive hyperplasia). Absorbance centered around 1236–1242 cm\(^{-1}\) is relatively specific for collagen and nucleic acids,\(^{16}\) suggesting that the increased \(A_{1200-1700}\) observed is primarily due to collagen (having controlled for relative amounts of RNA and DNA with 1121/1020 index). Collagenous scarring is a major product of chronic inflammation. A greater degree of inflammatory activity would be expected within an inflamed lymph node reacting to a foreign microbe than within a low-grade lymphoma, the latter being comprised of lymphocytes that would be expected to appear relatively normal to the host immune system.

**Comparison with Leukemia Study**

Recent study of chronic lymphocytic leukemia (CLL) has shown a lack of a significant 1121-cm\(^{-1}\) (RNA) pulse in CLL cells as noted by Schultz et al.\(^{22}\) This observation is consistent with CLL being a chronic indolent disease characterized by accumulation of poorly functioning cells due to failure of apoptosis rather than being a primarily proliferative disorder.

The \(A_{1240}/A_{1084}\) ratio is the same for both normal lymphocytes and CLL cells\(^{22}\) and approximates that of pure nucleic acids,\(^8\) despite greater amide I and amide II protein absorbance relative to nucleic acid phosphodiester absorbance in normal lymphocytes compared with CLL cells. This observation further supports the specificity of the amide III band at 1242 cm\(^{-1}\) for collagen and suggests that when collagen is not present, the phosphodiester stretching bands are primarily due to DNA and RNA absorbances.

Case 7 is one of CLL that progressed to small lymphocytic lymphoma (SLL). We observed a distinctly lower lipid content relative to nucleic acid content in case 7 (SLL) compared with the high-grade lymphomas and the other low-grade lymphomas (Fig. 8), in keeping with similar findings by Schultz et al.\(^{22}\) for CLL.

**Lipid Effects**

With the exception of case 7, differences in lipid content as judged by the intensity of absorbance in the lipid region (2800–3000 cm\(^{-1}\)) relative to the nucleic acid region centered around 1084 cm\(^{-1}\) show no trend with respect to grade (Figs. 1, 2, and 8). In fact, differences in these relative intensities between same graded cases, and within cases, are much larger than the average difference between high and low grade (Fig. 8), which is trivial in comparison. Therefore, lipid absorbances are unlikely to have been a factor in the observed 1121/1020 trend with lymphoma grade. The magnitude of the absorbance at 1084 cm\(^{-1}\) by adenocarcinoma cell extracted lipids\(^6\) is about half that of the C==O band at 1740 cm\(^{-1}\) (evident in Figs. 1 and 2 on the high frequency shoulder of the amide I band) that in turn is very small compared with the cluster of lipid bands at 2800–3000 cm\(^{-1}\). Case 6 was among the highest in lipid content, whereas case 7 was the lowest (Fig. 2). The lipid variation within our cases does not appear to be responsible for the 1121-cm\(^{-1}\) pulse and 1121/1020 index effects that we observed. More extreme variations in lipid, however, could potentially have a larger impact on the 1121/1020 index.

**CONCLUSIONS**

On the basis of infrared absorbances due to nucleic acids, FTIR spectroscopy can distinguish be-
between clinically aggressive (high-grade) and clinically indolent (low-grade) lymphomas. The ratio \( A_{1121}/A_{1020} \) rises with lymphoma grade, and a characteristic 1121-cm\(^{-1}\) centered absorbance pulse appears, attributable to increasing RNA content. Absorptances due to collagen and glycogen in the region of the phosphodiester stretching bands (900–1350 cm\(^{-1}\)) are readily identified and could be interactively subtracted, leaving an absorbance profile in this region that is primarily due to a combination of DNA and RNA. The absorbance profile moves closer to that of RNA and further from that of DNA with increasing lymphoma clinicopathological grade, and this trend could potentially form the basis of a universal cancer grading parameter (assuming standardized spectroscopic methods).

The ratio \( A_{1240}/A_{1084} \) also rises with lymphoma grade and is an index of the collagen-to-nucleic acid ratio. This trend is opposite to that seen for many epithelial cancers (an exception is breast cancer\(^{17}\)) and may indicate the degree of host inflammatory reaction to a malignancy.

Spectra of inflammatory tumors show changes in the region of the SPSB similar to those of malignant tumors, further suggesting that these changes are due to the functional or biosynthetic activity of the cells and not something specific to malignancy. Inflammatory tumors also show greater \( A_{1200–1700} \) absorbance attributable to collagen than low-grade lymphomas, and this difference may have value in the diagnostic distinction between benign reactive and low-grade malignant lymphoma.

The infrared spectral trends described here may potentially be applied to discrete areas within tissue sections or cell preparations using the FTIR microscope, allowing correlation with morphology and a high degree of spatial resolution.

The potential to simultaneously quantify relative amounts of protein, RNA, and DNA raises the possibility that FTIR may be used to analyze tumor growth perturbation induced by drugs or other therapies. Other applications may exist in the study of inflammatory disorders or in the study of cell aging.

REFERENCES


