INTERNAL MEDICINE - PEDIATRICS

RELATIONSHIP BETWEEN KUPFFER CELLS, INFLAMMATION, AND FIBROSIS IN CHRONIC HEPATITIS B AND C

N. Stănculeţ¹, Adriana Grigoraş¹, Roxana Avădanei¹, Alina Floarea-Strat², Cornelia Amălinei¹, Irina-Draga Căruntu¹
University of Medicine and Pharmacy “Grigore T. Popa”- Iaşi
Faculty of Medicine
1. Discipline of Histology
Clinical Hospital of Infectious Diseases “St. Parascheva” Iaşi
2. Pathology Laboratory

RELATIONSHIP BETWEEN KUPFFER CELLS, INFLAMMATION, AND FIBROSIS IN CHRONIC HEPATITIS B AND C (Abstract): Kupffer cells are liver parenchymal components of the immune system able of intervention in pathogenic mechanisms involved in viral hepatitis and tumoral lesions. Our study aimed to evaluate the Kupfferian hyperplasia in chronic hepatitis B and C, and to correlate with the severity of liver lesions, focusing on the modality in which Kupffer cells modulate the necroinflammatory events and fibrosis specific for chronic hepatitis morphologic substrate. We investigated 33 cases with chronic hepatitis B and 38 cases with chronic hepatitis C, diagnosed according to Ishak score (in chronic hepatitis B and C) and METAVIR score (in chronic hepatitis C). Kupffer cells were immunohistochemical labeled, by using an anti-CD68 antibody and heat-induced epitope retrieval (HIER) technique. The sinusoidal reaction expressed by CD68 (+) cells hyperplasia progressively increases along with the intensity of necroinflammatory activity and with the amplitude of fibrosis lesions. Statistically significant differences between Kupffer cells number and the degree of necroinflammatory activity and fibrosis, respectively (Ishak score) have been identified in both types of chronic hepatitis. However, no significant differences have been registered when comparing the Kupffer cells number corresponding to each degree of necroinflammatory activity and of fibrosis, in chronic hepatitis B versus C, respectively. Our results demonstrate the relationship between Kupffer cells and the severity degree of the disease, without differences between chronic hepatitis B and C. Consequently, we may appreciate that the chronic hepatitis C specific lesions progression, different from that of chronic hepatitis B, is influenced by a different behavior of Kupffer cells, and not by their effective number. Keywords: KUPFFER CELLS, CD68, CHRONIC HEPATITIS

A highly represented liver cells population had been firstly described in 1876, by Karl Wilhelm von Kupffer which he had named "Sternzellen" or stellate liver cells corresponding to their shape (1). These cells are considered as an intrinsic part of liver sinusoids endothelial cells and furthermore directly derived from them (1). Tadeusz Browicz had correctly defined the macrophagic characteristics of the stellate liver cells, in 1898; only after two decades researchers had named them as Browicz-Kupffer cells or simply Kupffer cells (2, 3). The blood that transits the liver is exposed
to approximately to $1.2 \times 10^7$ Kupffer cells/tissue grams (4).

Kupffer cells are the main phagocytic cells and APC in liver sinusoid, being at the same time important sources of cytokines exhibiting locally chemotactic action or of stimulatory activity of endothelial and Ito cells. Classic studies have revealed the existence of Kupfferian population heterogeneity and consequently, in a normal liver status, Kupffer cells from the periportal region are larger and more actively involved in phagocytosis, while those from the centrilobular region are smaller and more actively involved in cytokine production and in cytotoxicity (5, 6). A deeper insight into these data have been added by recent immunological studies identifying 2 main types within Kupfferian cell population, namely a proinflammatory phenotype (M1) and another involved in resolution of the inflammatory phenomena in the liver parenchyma (M2) (7, 8).

Classically, light microscopy studies have revealed Kupffer cell hyperplasia in liver sinusoids during the sequence of immunological events characteristics for chronic viral hepatitis (9, 10). Although standard light microscopy reveals the Kupffer cells growth in liver sinusoid and even the „cells crowding” in specific territories, the precise appreciation of the intensity of this phenomenon could be only achieved by supplementary examinations, such as immunohistochemistry.

The purpose of our study is that of evaluation of the Kupfferian hyperplasia in viral chronic hepatitis with B hepatitis virus (HBV) and C hepatitis virus (HVC), respectively, and correlation of Kupfferian hyperplasia with the severity of liver lesions, focusing on the modality in which Kupffer cells modulate the necro-inflammatory events and fibrosis specific for chronic hepatitis morphologic substrate.

**MATERIAL AND METHODS**

The study group comprised 71 cases of chronic viral hepatitis (subgroup 1 – 33 cases with chronic hepatitis B, subgroup 2 – 38 cases with chronic hepatitis C), diagnosed in the Histopathology Laboratory of the Clinical Hospital of Infectious Diseases “St. Parascheva” Iași.

The cases selection has been achieved starting with the histopathologic diagnosis made according to Ishak score (in chronic hepatitis B and C) and METAVIR score (in chronic hepatitis C), respectively, having as a main criterion the frame into a severity class according to mild, moderate, and severe necroinflammatory activity (NIA). Thus, subgroup 1 included 10 cases characterized by mild necroinflammatory activity (score 1-6), 17 cases with moderate necro-inflammatory activity (score 7-10), and 6 cases with severe necroinflammatory activity (score 11-18), and subgroup 2: 11 cases with mild necroinflammatory activity (score 1-6), 18 cases of moderate necro-inflammatory activity (score 7-10), and 9 cases of severe necroinflammatory activity (score 11-18).

The studied group has been investigated by immunohistochemistry using an anti-CD68 antibody and heat-induced epitope retrieval (HIER) technique. The working protocol included: blockage of the endogenous peroxidase, incubation with the anti-CD68 antibody (clone KP1, Novocastra, Leica Biosystems) with a dilution ratio of 1:100, amplification with the secondary antibody and the Streptavidin–Biotin-Peroxidase HRP complex (Novocastra Novolink, Leica Biosystems), and counterstain with Mayer’s Hematoxylin.
The quantitative evaluation has been performed by two independent examiners, on 5 microscopic fields/case, using x 200 magnification. After the quantification of CD68 positive cells number for all examined areas, the median value obtained has been associated to the following scoring system (13, 14, 15): CD68 positive cells number < 10 cells – score 0; 10-49 CD68 positive cells number – score 1; 50-99 CD68 positive cells – score 2; ≥100 CD68 positive cells – score 3.

Student’s t-test was used for all statistical comparisons.

RESULTS

Qualitative evaluation

The qualitative evaluation of CD68 positive cells distribution in sinusoids capillaries allowed the identification of the following aspects:

- the presence of a reduced number of CD68 positive cells, localized mainly in periportal sinusoidal spaces in patients diagnosed with mild activity type of chronic liver disease (fig. 1);

- the tendency of extension to the entire liver lobule and of CD68 focally concentration in specific “hyperplasia areas” in sinusoids, as closely tighten events to necroinflammatory lesions and portal and intralobular fibrosis enhancement (fig. 2).

Quantitative evaluation

The numerical results of the quantitative evaluation of Kupffer cells (median ± SD), related to the components of Ishak score (in both types of hepatitis) and of METAVIR score (in chronic hepatitis C), are summarized in table I and II.

Correlated to the numerical value correspondent to median and SD, the correspondent Kupffer cells score had, in both types of hepatitis, an ascendant trend related to the necroinflammatory activity (mild type - score 1 (24.54 ± 7.94), moderate type - score 2 (83.45 ± 28.44), and severe type - score 3 (126.45 ± 8.28)).

Differences between hepatitis B and C have been registered regarding the correlation to fibrosis (considering 1 and 2 score values for mild fibrosis and score values 3 and 4 for severe fibrosis). Thus, in chronic hepatitis B, Kupffer cells score had value 1 (31.96 ± 14.67) in mild fibrosis and value 3 (107.96 ± 18.87) in severe fibrosis, while in chronic hepatitis C mild fi-
Relationship between Kupffer cells, inflammation, and fibrosis in chronic hepatitis B and C

Fibrosis has been associated to a Kupffer cells score value of 1 (33.51 ± 14.69), and severe fibrosis - with a score of 2 (94.05 ± 31.37).

Related to Metavir score, exclusively applicable in chronic hepatitis C, we have noted the same increase of Kupffer cells number, thus cases with score A1F1/A1F2 being correlated to score 1 (30.64 ± 10.09), for cases with score A2F1/A2F2, Kupffer cells score being 2 (76.89 ± 18.36) and for cases with A3F3 –3 (119.62 ± 15.62).

**TABLE I**
CD68 positive cells in correlation with the Ishak (NIA, fibrosis) scoring system – chronic hepatitis B

<table>
<thead>
<tr>
<th>Cases (total #)</th>
<th>NIA score</th>
<th>CD68 cells (median ± SD, score)</th>
<th>Fibrosis score</th>
<th>CD68 cells (median ± SD, score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mild</td>
<td>4</td>
<td>24.54 ± 7.94</td>
<td>1</td>
<td>31.96 ± 14.67</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 Moderate</td>
<td>7</td>
<td>83.45 ± 28.44</td>
<td>2</td>
<td>107.96 ± 18.87</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6 severe</td>
<td>11</td>
<td>126.45 ± 8.28</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE II**
CD68 positive cells in correlation with the Ishak (NIA, fibrosis) and the METAVIR scoring systems – chronic hepatitis C

<table>
<thead>
<tr>
<th>Cases (total #)</th>
<th>NIA (score)</th>
<th>CD68 cells (median ± SD, score)</th>
<th>Fibrosis score</th>
<th>CD68 cells (median ± SD, score)</th>
<th>Metavir score</th>
<th>CD68 cells (median ± SD, score)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11 mild</td>
<td>2</td>
<td>30.64 ± 10.09</td>
<td>1</td>
<td>33.51 ± 14.69</td>
<td>A1F1</td>
<td>30.64 ± 10.09</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td></td>
<td>1</td>
<td></td>
<td>A1F1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td></td>
<td>2</td>
<td></td>
<td>A1F1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>94.05 ± 31.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18 moderate</td>
<td>7</td>
<td>86.79 ± 21.44</td>
<td>2</td>
<td>33.51 ± 14.69</td>
<td>A2F1</td>
<td>76.89 ± 18.36</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td></td>
<td>3</td>
<td></td>
<td>A2F2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td></td>
<td>3</td>
<td></td>
<td>A2F2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>3</td>
<td></td>
<td>A3F3</td>
<td></td>
</tr>
<tr>
<td>9 severe</td>
<td>11</td>
<td>122.88 ± 16.83</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td></td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Correlations between Kupffer cells and NIA – Ishak score**
Statistically significant differences between Kupffer cells number corresponding to the 3 degrees of necro-inflammatory activities within mild versus moderate (p <
0.001), mild *versus* severe (p < 0.001), and moderate *versus* severe (p < 0.001) have been identified by Student t test, in both types of chronic hepatitis.

However, no statistically significant differences have been identified when comparing the Kupffer cells number corresponding to each degree of necroinflammatory activity and chronic hepatitis B *versus* C.

**Correlations between Kupffer cells and fibrosis – Ishak score**

In both types of chronic hepatitis, t Student test revealed the existence of significant differences between Kupffer cells number corresponding to the two grades of fibrosis, mild *versus* severe (p < 0.001). No statistically significant differences have been identified when comparing the Kupffer cells number corresponding to mild and severe fibrosis, in chronic hepatitis B evaluation versus chronic hepatitis C (p < 0.001).

**Correlations between Kupffer cells and METAVIR score**

Statistical analysis based on Metavir score, applied only in chronic hepatitis C revealed significant differences between Kupffer cells number corresponding to cases evaluated by A1F1/A1F2 score versus A2F1/A2F2 score versus A3F3 score (p < 0.001).

**DISCUSSION**

**Kupffer cells and necroinflammatory activity – Ishak score**

Kupffer cells are liver parenchyma components of the immune system able of intervention in pathogenic mechanisms involved in viral hepatitis and tumoral lesions, by their capacity to phagocyte antigens and to produce a panel of cytokines (16, 17, 18). They represent 15% of liver cell population and 80-90% of resident macrophages of human body (19).

Literature studies describe two mechanisms responsible for the conservation of Kupffer cells normal number in liver parenchyma in physiological conditions: attraction of new monocytes in liver sinusoids and their transformation into Kupffer cells and the multiplication of already existent Kupffer cells (19, 20).

Kupffer cells exhibit an intense phagocytic activity in liver parenchyma, composing an antigen filter for substances spread by portal vein from the digestive tract. The phagocytic activity is sustained by inflammatory cytokines synthesis, such as: IL-1, IL-6, IL-12, TNF, GM-CSF, CXCL9, CXCL10, MIP-1 (macrophage inflammatory protein 1 alpha), and RANTES (regulated on activation, normal T-cell expressed and secreted) (20, 21, 22). Moreover, activated Kupffer cells express on their surface a series of molecules that provide them similar features with APC, as CD80 and CD40, in viral liver pathology (23).

Nowadays, the idea of cellular heterogeneity within Kupfferian cell population is increasingly supported not only by classic relation between dimension and location in liver sinusoids, as larger and more actively phagocytic periportal Kupffer cells as compared to centrilobular ones, the latter being smaller and especially involved in cytokines synthesis (19, 22, 24). A recent experimental study is registered in this direction, its results identifying at least two Kupfferian subpopulations, as following: CD68 (+) showing an intense phagocytic activity and CD11b (+) exhibiting the capacity of production of immunostimulatory cytokines (such as TNF and IL-12) (22). Supplementary, the hypothesis that CD68
Kupffer cells might basically constitute the resident (fixed) liver Kupfferian cells population, while CD11b (+) cellular subtype might represent a migrated population of macrophage-type cells of spleen and bone marrow origin, mainly in pathologic conditions, such as inflammatory conditions (7, 25) is currently accepted.

Unfortunately, the data regarding these cells roles in viral chronic hepatitis, both with HVB and with HVC are far from being elucidated until present (12, 26).

Within this “collective effort” expressed by a multitude of studies spread by literature concerning complete phenotypic characterization of Kupffer cells, mainly in viral chronic liver pathology, our study focused on CD68 immunostaining of the existent cellular population of liver sinusoids, in 71 cases of chronic viral hepatitis, is currently framed.

Our results based on immunohistochemical staining and Kupffer cells quantification in chronic hepatitis B and C, respectively, according to the degree of necroinflammatory activity, revealed the statistically significant increase of Kupffer cells number (p < 0.001) starting from the mild to moderate, and severe necroinflammatory activity, respectively. It’s extremely interesting that the statistical analysis did not reveal significant differences between the numbers of Kupffer cells corresponding to each specific type of necroinflammatory activity (mild, moderate, and severe) analysis, when comparing viral hepatitis B with viral hepatitis C. Considering the pathogenic mechanism, this result may indicate the fact that the different evolution of the two types of hepatitis is not correlated to Kupffer cell number but to their specific behavior of Kupffer cells corresponding to the pathogenic agent.

Our own observations, based on Kupffer cells numerical value and their afferent score, are in agreement to literature data. Qualitative or quantitative researches aimed to highlight the sinusoidal reaction in chronic viral hepatitis, reflected in Kupffer cells number, have pointed out their progressive numerical amplification starting even from mild necroinflammatory activity types (27, 28).

The specific correlation between Kupffer cells and lymphocytes in sinusoids and the direct correlation between their number and the hepatocyte necrosis lesions intensity have been some of the first morphological aspects observed in light microscopy. Thus, McGuiness and al., by comparing CD68 (+) Kupffer cells number in 10 cases of chronic hepatitis, observed an increase of their number associated to a dispersion into the liver lobule, in comparison to control cases (24).

The Kupfferian hyperplasia is far more important as viral chronic hepatitis has been appreciated with a higher aggressiveness. Quantitative analysis of the area occupied in liver parenchyma by immunolabeled Kupffer cells confirms the results of the study. The authors observed a progressive increase in the percentage occupied by Kupffer cells in total area of liver surface correlated to disease severity, ranging from 2.51 ± 0.88 in moderate activity and 4.71 ± 2.23 in severe activity types of chronic hepatitis (29).

The involvement of Kupffer cells in Ito cells activation and in fibrillogenic activity in liver parenchyma have been unanimously recognized (8, 30, 31). However, the molecular aspects of interactions between activated liver macrophages and myofibroblasts are still incompletely known, repre-
senting a point of interest directed toward identification of new possible therapeutic targets in liver fibrosis (32).

**Kupffer cells and fibrosis – Ishak score**

Recent studies have shown that HVB chronic liver infection even in cases exhibiting a mild necroinflammatory activity is associated to sinusoidal and portal collagen synthesis development. This phenomenon is attributed to Kupffer cells reactivity even in mild aggressiveness hepatitis types to produce a panel of pro-fibrillagenic cytokines, such as TGF-β1 (33).

It is unanimously accepted that patients with HVC have more severe fibrosis lesions compared to HVB chronic hepatitis patients (34). Our results confirmed this assertion.

The evaluation of the fibrosis severity correlated to the necroinflammatory activity has revealed more important lesions in chronic hepatitis C. Compared to chronic hepatitis B, more cases with fibrosis of score 2 and 3 showed mild necroinflammatory activity. Compared to chronic hepatitis B, no case showed score 1 fibrosis and score 4 fibrosis was detected in moderate necroinflammatory activity. All cases showing severe necroinflammatory activity had fibrosis score 4, when compared to chronic hepatitis B.

We have registered statistically significant differences between Kupffer cells number associated to cases with fibrosis score 1 and 2, and score 3 and 4, respectively, in each hepatitis type (p < 0.001). We have also noted that the score value correlated to Kupffer cells expression increased from 1 to 3 in chronic hepatitis B and from 1 to 2 in chronic hepatitis C, corresponding to mild and severe fibrosis, respectively. However, despite the above mentioned fibrosis score in the same intensity of the necroinflammatory activity, statistical analysis revealed the lack of statistically significant differences between chronic hepatitis B and C, correlated to Kupffer cells number in fibrosis score 1 and 2 fibrosis score and 3 and 4, respectively.

This negative result supports the idea of fibrosis development as an event not necessarily related to Kupffer cells. The dramatic changes of the last 10-15 years concerning liver fibrosis have been closely correlated to the deciphering of the extracellular matrix dynamic and ultrastructure, to the elucidation of the liver myofibroblasts biology and, not the last, the integration of all these new elements in the complex process nowadays known as “connective repair” (the integrated wound healing response) (35, 36).

**Kupffer cells, necroinflammatory activity, and fibrosis – Metavir score**

Although Metavir score assures a specific and reproducible evaluation in C viral hepatitis (37), being largely used in histopathologic diagnosis formulation, the mainstream publication review revealed the complete lack of the researches oriented towards the relationship between Kupffer cells and lesions severity, corresponding to Metavir score categories in this type of hepatitis. To our knowledge, there is only a single study that quantifies Kupffer cells and is analyzing their dynamic related to the F (fibrosis) parameter of Metavir score, in the pathologic context of chronic hepatitis induced by chronic alcohol consumption and of fibrosis that develops in the evolution of this disease (38). The mentioned study indicates the lack of a direct correlation between CD68 expression, as a marker
of Kupffer cells, and the degree of severity of the disease, progressively from stage I to III (38).

Within this context we consider that our data have a degree of novelty supported by the differences obtained by comparison of the results obtained by the use of the two scores, Ishak and Metavir. In our opinion, the 3 classes of severity A1F1/A1F2, A2F1/A2F2, and A3F3 of Metavir score more likely reflect the lesions evolution, including the correlation between the necroinflammatory activity and fibrosis than the severity classes separately defined by necroinflammatory activity and fibrosis, respectively, in Ishak score. The most convincing argumentation is provided by the 4 cases that, according to Metavir score, have been framed into a higher category of severity, by A3F3 score. The statistically significant differences between Kupffer cells number (median and SD) in all 3 classes of Metavir severity indicate the involvement of Kupffer cells in enhancement of both inflammation and fibrosis, during the lesions severity evolution in C hepatitis.

In our current activity, the additional use of Ishak score in chronic hepatitis C is justified by the fact that the large numerical value scale correspondent to necroinflammatory and fibrosis activities evaluation offers more precise criteria to evaluate the status of liver parenchyma involvement at the diagnosis moment and METAVIR score, simultaneously applied, allows an evaluation of the general histologic activity, including the interface hepatitis and of associated components of lobular necrosis.

As a consequence of the study oriented towards the quantitative profile of Kupffer cells, we may appreciate that Metavir score offers, by numerical values, an image of the inflammatory process and of fibrillogenesis that includes, as a pathogenic substrate, including Kupffer cells involvement.

CONCLUSIONS
The sinusoidal reaction in chronic liver diseases of viral etiology expressed by CD68 (+) cells hyperplasia progressively increases along with the intensity of necroinflammatory activity and with the amplitude of fibrosis lesions. The classic microscopically findings, the quantitative evaluation, and the statistical analysis indicate the involvement of Kupffer cells, directly correlated to the severity degree of the disease, without differences between B and C chronic hepatitis. Consequently, we may appreciate that the chronic hepatitis C specific lesions progression, different from that of chronic hepatitis B, is influenced by a different behavior of Kupffer cells, and not by their effective number.

REFERENCES
Relationship between Kupffer cells, inflammation, and fibrosis in chronic hepatitis B and C


**NEWS**

**SIGNET-RING CELL MELANOMA OF THE GASTROESOPHAGEAL JUNCTION**

Malignant melanomas are well-known for demonstrating a wide range of microscopic appearances and, therefore, simulate other tumor types. Consequently, melanomas with unusual phenotypes may present a diagnostic challenge to the surgical pathologist. Signet-ring cell melanoma of the gastroesophageal junction is the first case of probable primary signet-ring cell melanoma at this site and the second case reported in the gastrointestinal tract. Signet-ring cell melanoma is defined as a melanoma with more than 50% of tumor cells having signet-ring–shaped nuclei because of compression by intracytoplasmic globules. Signet-ring cell morphology is extremely rare in melanomas (0.5%) and is one of 5 unusual variants described in a review of diversity in melanomas by Nakhleh et al. Signet-ring cell melanoma was first described in 1988 by Sheibani and Battifora. In 2 reported cases, transmission electron microscopy was used to identify melanosomes, premelanosomes, and discrete aggregates of intermediate filaments distending the cytoplasm. The signet-ring cells in each of these cases strongly expressed S100 protein, HMB-45, and vimentin, confirming the diagnosis. Since the entity was first described, only 19 additional cases have been reported in the literature. Approximately one-half of those cases were metastases or recurrences, with only 8 cases reported as primary lesions. The diagnosis can be confirmed by the presence of immunoreactivity for melanocytic markers, the absence of mucin and immunohistochemical staining for pancytokeratin, and if necessary, examination for ultrastructural features including melanosomes, in signet-ring cell morphology tumors. (Melissa A, Grilliot, R. Goldblum, Xiuli Liu. Signet-Ring Cell Melanoma of the Gastroesophageal Junction. Arch Pathol Lab Med. 2012;136:324–328).

Doina Butcovan