Leiomyoma of the Gastrointestinal Tract with Intracytoplasmic Inclusion Bodies

Report of Three Cases

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Summary

We report three cases of leiomyoma of the gastrointestinal tract with intracytoplasmic inclusion bodies similar to those characteristic of inclusion body fibromatosis (IBF). The first two cases represent leiomyoma of the stomach: one in a 70-year-old female and the other in a 72-year-old female. In both instances inclusion bodies were present in a large amount. In the third case the leiomyoma was located in the esophagus of a 63-year-old male and inclusion bodies in this case were rare. In all three cases an immunohistochemical analysis showed positivity of the tumor cells for muscle specific actin HHF35 (MSA), α-smooth muscle actin (SMA), h-caldesmon and desmin. The first case showed some inclusion bodies with positivity for cytokeratin CAM 5.2 and focal weak positivity for cytokeratin 18. In the second case the inclusion bodies were positive at the periphery with antibodies directed against MSA and SMA. In the third case the inclusion bodies were immunohistochemically entirely negative. Ultrastructurally, the inclusion bodies in the first case were composed of aggregated filaments, some with entrapped cytoplasmic organels and others with finely granular dense cores.

Key words: leiomyoma- inclusion bodies- eosinophilic bodies- infantile digital fibromatosis

Souhrn

Leiomyom gastrointestinálního traktu s intracytoplazmatickými inkluzními tělísky. Popis tří případů

Popisujeme tři případy leiomyomu zažívacího traktu s inkluzními tělísky podobnými inkluzím vyskytujícím se u infantilní digitální fibromatózy. První dva případy jsou leiomyomy žaludku, v obou těchto případech byla tělíska přítomna ve velkém množství. Ve třetím případu jde o leiomyom jícnu s výskytem pouze ojedinělých tělísek. Imunohistochemické vyšetření prokázalo ve všech případech pozitivitu buněk leiomyomu s protilátkou proti aktinu HHF35, α-aktinu, h-caldesmonu a dezminu. Inkluzní tělíska byla v prvním případu fokálně pozitivní při průkazu cytokeratinů CAM 5.2 a CK 18. Ve druhém případu vykazovala tělíska periferní pozitivitu s protilátkou proti α-aktinu a aktinu HHF 35. Ve třetím případu byla tělíska zcela negativní. Elektronmikroskopické vyšetření jsme vzhledem k nedostatku materiálu provedli pouze u prvního případu. Ultrastrukturálně byla tělíska tvořena agregáty filament, v některých se zavzatými cytoplazmatickými organelami.

Klíčová slova: leiomyom – inkluzní tělíska – eozinofilní tělíska – infantilní digitální fibromatóza

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Inclusion body fibromatosis (IBF), synonymically infantile digital fibromatosis, is a distinctive type of fibromatosis characterized by the presence of eosinophilic inclusion bodies in the cytoplasm of myofibroblastic cells (5, 20). This type of fibromatosis occurs mostly in the digits of infants and young children. However, morphologically similar lesions are sometimes reported in extradigital sites in adults (4, 16). The pathogenesis of the eosinophilic inclusion bodies in IBF is believed to be related to abnormal aggregates of contractile actin filaments (5). Immunohistochemically, the inclusion bodies are reported to be actin positive or entirely negative (5, 12). One report has described a case of IBF located in the tongue with vimentin positive bodies (4). Similar bodies are rarely described in other lesions with myofibroblastic cells, including stromal cells in phyllodes tumors and fibroepithelial tumors of the breast and cervical polyps (2, 3, 8, 13, 19). We

have found two reports with a full description of leiomyoma with similar bodies, one located in the urinary bladder and the other intracerebrally (1, 10). We report three additional cases of leiomyomas with intracytoplasmic inclusion bodies, two of them located in the stomach and the third in the esophagus. In one case we found immunohistochemical positivity of the inclusion bodies with cytokeratin 18 and CAM 5.2. To date this finding has never been reported in any lesions with inclusion bodies similar to those of IBF.

Case Reports

Case 1

A 70-year-old female visited her physician because of pain in the upper abdomen. A gastroscopical examination revealed a submucous nodule located in the stomach antrum and a CT scan revealed a circumscribed oval tumor 30 mm in diameter. A partial gastrectomy was performed. Now, 24 months after the operation, the patients shows no signs of the disease.

Case 2

A 72-year-old female with multiple myeloma treated with chemotherapy and corticotherapy died because of a diverticle perforation in the sigmoid colon. The leiomyoma of the stomach was an incidental finding discovered during the autopsy.

Case 3

This case from our consultation files involved an esophageal submucosal tumor, 5 mm in diameter, in a 63-year-old male. This tumor, located in the esophageal resection margin, represents an incidental finding in a patient with diffuse adenocarcinoma of the stomach. Further anamnestical data are not available.

Materials and Methods

Sections from formalin-fixed, paraffin embeded tissue from all tumors were stained with hematoxylin-eosin, Masson's trichrome stain, Congo red and periodic acid-Schiff with and without pretreatment by amylase. Immunohistochemical staining was performed using the avidin-biotin-complex method with antibodies directed against following antigens: actin HHF35 (1:100, Dako, Glostrup, Denmark), α-actin (1:100, Dako), ß-actin (1:20000, Abcam Limited, Cambridge, UK), F-actin (1:200, Abcam Limited), cytokeratin CAM 5.2 (1:10, Becton-Dickinson, Mountain View, CA, USA), cytokeratin AE1/AE3 (1:50, Dako), cytokeratin 7 (1:25, Dako), cytokeratin 18 (1:50, Dako), cytokeratin 19 (1:50, Dako), cytokeratin 20 (1:50, Dako), CD 34 (1:200, Immunotech S.A., Marseille, France), cytokeratin 34ßE12 (1:50, Dako), MNF 116 (1:100, Dako), CD 117 (1:400, Dako), desmin (1:200, Dako), h-caldesmon (1:50, Dako), S-100 protein (1:400, Dako) and vimentin (1:300, BioGenex, San Ramon, CA, USA). Immunohistochemical staining with antibodies against α-smooth muscle actin (SMA) and actin HHF35 (MSA) was performed with and without incubation in 1% KOH in 70% ethanol at room temperature for 30 min. before pretreatment with 0.1% trypsin at 37 °C for 30 min. An electron microscopy examination was done on formalin-fixed, paraffin-embeded tissue only in the first case; in the second and third case we did not have enough material.

Results

Case 1

Grossly, the resected specimen consisted of part of the stomach wall on the external surface and intramurally with the tumor 30x30x25 mm. The tumor was circumscribed and white and it had a whorled trabecular appearance on the cut section. Microscopically, the tumor had the typical appearance of leiomyoma and it was composed of whorled, anastomosing fascicles of uniform fusiform smooth muscle cells. The nuclei were elongated with finely dispersed chromatin. In the cytoplasm of many tumor cells there were small, round or elongated inclusion bodies. These were basophilic or unstained in hematoxylin-eosin stained sections (Fig. 1a). They were PAS positive both with and without pretreatment with amylase (Fig. 1b), and unstained by Masson's trichrome and Congo red stain. Mitotic figures were not found. Focally, there were degenerative changes with hyaline fibrosis. The margin of the tumor was microscopically circumscribed. The tumor was located in the muscle layer and grew exophytically without extension into the mucosa or submucosa. The results of an immunohistochemical study are summarized in Table 1. The tumor cells were positive for MSA, SMA, ß-actin, F-actin, h-caldesmon, desmin and vimentin, but despite pretreatment with KOH in ethanol and trypsin the inclusion bodies were negative with the same antibodies (Fig. 1c). S-100 protein, CD 34 and CD117 were negative in both the tumor cells and the inclusion bodies. About 30% of the inclusion bodies showed positivity for cytokeratin CAM 5.2 (Fig. 1d) and about 20% displayed weak positivity for cytokeratin 18. Electron microscopy showed typi-

cal smooth muscle cells with myofilaments in parallel arrangement and with focal densities along their course. Many of the tumor cells contained intracellular, but not membrane-bound inclusions. Some of these inclusion consisted of an aggregation of fibrillar and granular material, while others had components of fibrils on the periphery of the inclusion with a dense granular core (Fig. 2). Degenerated cytoplasmic organels were entrapped in some of the inclusions.

Tab. 1. Immunohistochemical results

	Case No. 1		Case No. 2		Case No. 3	
	Tumor cells	Inclusions	Tumor cells	Inclusions	Tumor cells	Inclusions
[a]α-actin	+	-	+	+	+	-
[a]Actin HHF35	+	-	+	+	+	-
β-actin	+	-	NP	NP	NP	NP
F-actin	+	-	NP	NP	NP	NP
Desmin	+	-	+	[d]+-	+	-
Vimentin	+	-	+	-	+	-
H-caldesmon	+	-	+	-	+	-
S-100 protein	-	-	-	-	-	-
CD 117	-	-	-	-	-	-
Cytokeratin CAM 5.2	-	[b]+-	-	-	-	-
Cytokeratin AE1/AE3	-	-	-	-	NP	NP
Cytokeratin 34βE12	-	-	NP	NP	NP	NP
Cytokeratin MNF116	-	-	NP	NP	NP	NP
Cytokeratin 7	-	-	NP	NP	NP	NP
Cytokeratin 18	-	[c]-+	-	-	-	-
Cytokeratin 19	-	-	NP	NP	NP	NP
Cytokeratin 20	-	-	NP	NP	NP	NP
CD 34	-	-	-	-	-	-

a staining performed with and without pretreatment with KOH in ethanol and with trypsin
b positivity in about 30% of the inclusion bodies
c weak positivity in about 20% of the inclusion bodies
d weak positivity in about 15% of the inclusion bodies
NP = not performed
\+ = positive results
\- = negative results

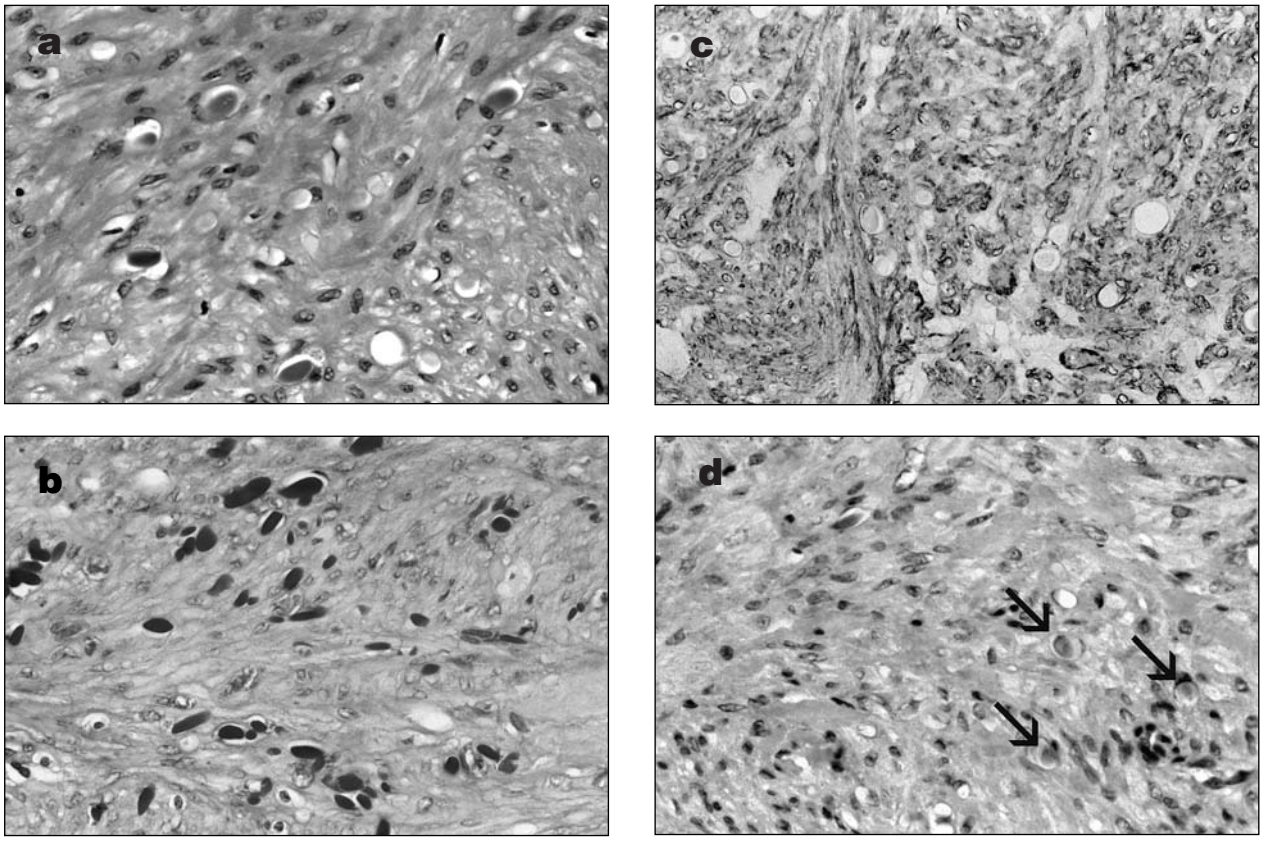

Fig. 1. Case No. 1 – typical areas of leiomyoma with multiple inclusion bodies which are basophilic in hematoxylin-eosin (a), PAS positive (b), actin negative (c), and cytokeratin CAM 5.2 positive (d). Original magnification 300x

Case 2

This tumor represents an incidental finding at the autopsy. The oval shaped circumscribed tumor 4x3x2 mm was located in the stomach wall. Microscopically, the tumor had the typical features of leiomyoma. In the cytoplasm of many tumor cells there were round or elongated inclusion bodies (Fig. 3a). These bodies were eosinophilic in hematoxylin-eosin stain, stained brightly red by Masson's trichrome, and were PAS and Congo red negative. Immunohistochemically, the tumor cells were positive for MSA, SMA, h-caldesmon and desmin. The inclusion bodies showed positivity on the periphery with antibodies against MSA and SMA (Fig. 3b). Some of the inclusion bodies were continuous with the cytoplasmic actin positive bundles. The extent and intensity of the positivity did not change after the pretreatment with KOH in 70% ethanol and trypsin. In addition, about 15% of the inclusions showed weak positivity for desmin. Due to the small size of the tumor we did not have enough material for a complete immunohistochemical and electron microscopic study. The results of the performed immunohistochemical examinations are summarized in Tab. 1.

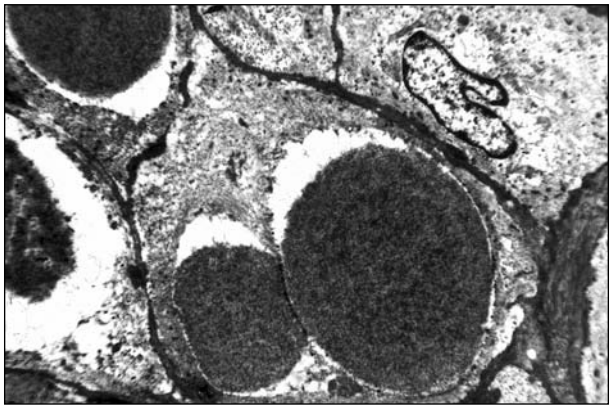

Fig. 2. Case No. 1 – ultrastructural appearance of intracytoplasmic inclusions composed of fibrilar and granular material. The clear spaces surrounding the inclusions represent artefacts. Original magnification 3600x

Case 3

This case was from our consultation files and we had only one block of paraffin-embedded tissue of the tumor. Microscopically, the tumor was located in the muscular layer of the esophagus and circumscribed without extension to the mucosa. As in the first two cases, the tumor had the typical features of leiomyoma. No mitoses were found. In the cytoplasm of some tumor cells there were rare small, round or elongated inclusion bodies. These were pale eosinophilic or unstained in hematoxylin-eosin stained sections. They were PAS positive both with and without pretreatment with amylase, stained weakly red by Masson's trichrome, and were Congo red negative. Only a limited amount of tissue was available for an immunohistochemical analysis and we did not have enough material for an electron microscopical study. The results of the performed immunohistochemical examinations are summarized in Table 1. The tumor cells showed positivity for MSA, SMA, desmin, vimentin and h-caldesmon. S-100 protein, CD 34 and CD 117 were negative. The inclusion bodies were entirely negative.

Discussion

Intracytoplasmic inclusion bodies are a well-known feature of various neoplasms. Ultrastructurally, they are composed of aggregated filaments. The types of these filaments vary in different lesions. Both cytokeratin and vimentin could be detected in a rhabdoid tumor, or cells with rhabdoid morphology in various other tumors (6, 9, 15, 17). Aggregated actin filaments are typically seen in IBF. However, on rare occasion inclusions similar to those found in IBF have been described in other lesions, including phyllodes

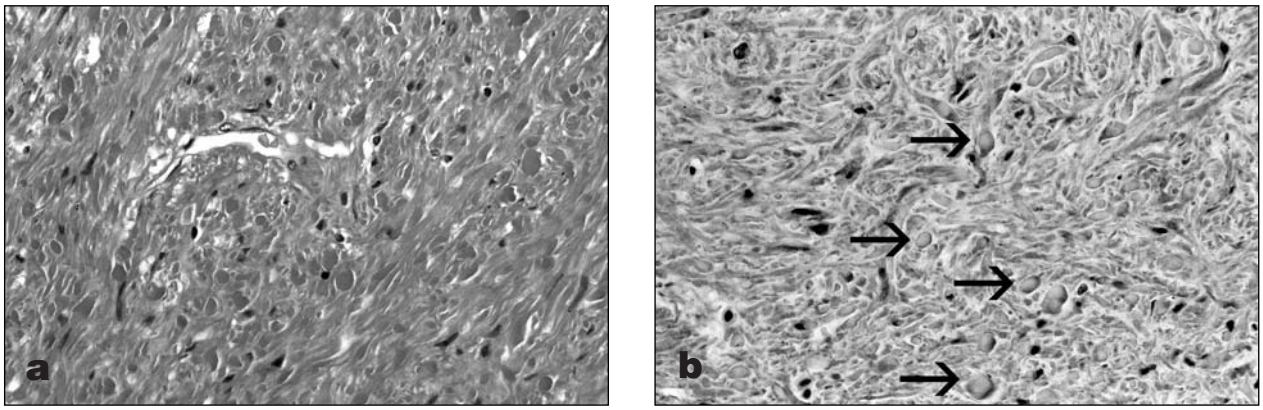

Fig. 3. Case No. 2 – leiomyoma with multiple inclusions which are eosinophilic in hematoxylin-eosin stain (a) and α-actin positive on the periphery (b). Original magnification 200x

tumors (8), fibroepithelial tumors of the breast (3,13), endocervical polyps (2,19) and two leiomyomas (1,10). Recently, a short report described 30 cases of colorectal leiomyomas, 18 of which contained multiple eosinophilic globules (11). The authors found immunohistochemical positivity of the globules for actin and desmin in all cases. These cases are not fully documented, but they probably represent leiomyomas with inclusions similar to those found in IBF and other lesions. However, the inclusion is not evident on their electron microscopy figure and there is only one other figure of hematoxylin-eosin stained tissue.

The eosinophilic inclusion bodies similar to those found in IBF are located in the cytoplasm of actin-rich cells, including myofibroblasts and smooth muscle cells. Various immunohistochemical studies of IBF showed actin positivity in the inclusions in some studies, but not in others (5,12). Actin positivity was usually demonstrated on alcohol- fixed specimens, whereas formalin- fixed specimens were negative or showed actin positivity on the periphery of the inclusions. However, actin positivity was demonstrated in the inclusions in formalin- fixed specimens that were pretreated with KOH in 70% ethanol and trypsin (12). This phenomenon could be caused by the direct effect of aldehyde fixatives such as formalin. It is partly irreversible because of permanent changes in the antigenic determinant; another part is being reversible because it is due to steric hindrance based on intramolecular crosslinkage in the protein molecules containing the antigenic determinant. This steric hindrance should be removed by pretreatment with proteinases such as trypsin, but in spite of this procedure some cases still showed negative immunohistochemical staining of the inclusion bodies. This is believed to be caused by excessively densely accumulated or degradated actin filaments and/or direct formalin-induced damage.

The pathogenesis of the eosinophilic inclusion bodies in IBF and leiomyoma is believed to be related to abnormal aggregates of contractile actin filaments (5). This theory is supported by the ultrastructural and immunohistochemical findings. However, one report of IBF located in the tongue described positivity of the inclusion bodies for vimentin and suggest that other types of abnormally aggregated filaments could give rise to intracytoplasmic inclusion bodies (4). In the first of our cases, some of the inclusion bodies showed moderate immunohistochemical positivity for cytokeratin CAM 5.2 and weak positivity for cytokeratin 18. In addition, the inclusions were PAS positive and unstained by Masson's trichrome. In the second case the inclusions were actin positive and stained brightly red by Masson's trichrome. They were also PAS negative. In the third case the inclusion bodies were immunohistochemically entirely negative, stained weakly red by Masson's trichrome and were PAS positive. The different immunohistochemical results and histochemical staining of the inclusion bodies may be caused by the varying degree of accumulation or degredation of the actin filaments and/or by the presence of other components in the inclusions. Several reports have described expression of cytokeratin in smooth muscle and smooth muscle tumors (7,14). Immunoreactivity was demonstrated in both frozen sections and paraffin- embedded tissue. In the former the positivity was strong. In the latter, however, the staining was usually focal and in a dot- like pattern. In the first of our cases the tumor cells showed positivity for MSA, SMA, desmin, vimentin and h-caldesmon; but cytokeratin CAM 5.2 and 18 were positive only in the inclusion bodies. Monoclonal antibody CAM 5.2 reacts with cytokeratin peptides 8 and 18. These results could possibly represent false positivity probably due to cross-reactivity with other filaments. However, none of the other filaments could be immunohistochemically demonstrated in the inclusion bodies and we can not rule out the possibility that - in this our case - the inclusions are at least partly composed of cytokeratin intermediate filaments, most probably cytokeratin 8 and 18. These 52k Dalton and 45k Dalton cytokeratin peptides are found in most epithelial cells with the exception of stratified squamous epithelium.

The presence of eosinophilic intracytoplasmic inclusion bodies are a characteristic and diagnostic feature of IBF (20). In other lesions it is a morphologically interesting finding, but without prognostic significance. We should be aware of the possibility of these inclusions in leiomyomas of the gastrointestinal tract to avoid misdiagnosis of skenoid fibres occurring in gastrointestinal stromal tumors. Skenoid fibres are, however, composed of collagen and located extracellularly (18).

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RECENZE

Bartůňková, J.: **Imunodeficience.**
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Monografie vyšla sice před čtyřmi lety, ale protože žádná podobná přehledná kniha u nás ještě nevyšla, dovoluji si na ni upozornit.

V úvodu autorka velmi názorně opakuje starší i aktuální poznatky o fyziologii imunitního systému na molekulární úrovni a o vzájemné souhře humorálních a buněčných mechanismů nespecifické i specifické imunity. V obecné části považuji za poučnou kapitolu o ontogenezi imunitního systému. Jde o překvapivě zajímavý přehled jednotlivých period rozvoje hematopoezy a imunity od embryonálního až do postnatálního období.

Primární imunodeficience (dále ID) jsou přehledně tabelárně seřazené do skupin humorální ID, buněčné a kombinované ID, poruchy fagocytózy, poruchy komplementárního systému, syndromy se zvýšenou lomivostí chromosomů a defekty v apoptóze lymfocytů. Všeobecný patolog se s většinou chorob zařazených do uvedených skupin nikdy nesetká, aniž je podrží v paměti. Přehledné upořádání kapitoly však lehce poskytne informaci.

Na rozdíl od primárních ID se ve své praxi častěji setkáváme s důsledky ID sekundárních, na kterých se do značné míry podílejí léčebné postupy. Jde o dřeňové útlumy nebo poruchy počtu a funkce periferních leukocytů, zejména při uplatnění autoimunních mechanismů.

Sekundární protilátkové ID jsou způsobeny ztrátami imunoglobulinů z plazmy nebo jejich sníženou tvorbou. Ke ztrátě Ig dochází při proteinurii a při úniku GI traktem, při různých krevních ztrátách i při rozsáhlých popáleninách. ID při chorobách jater je spojena se sníženou proteosyntézou (složek komplementu, proteinů akutní zánětlivé fáze). Při diabetu jde naopak o deficit buněčné imunity.

Autorka dále přehledně pojednává o patogenetických souvislostech ID v důsledku léčby, a to nejen v transplantologii, ale též v chirurgii a intenzivní medicíně. Není vynechaná ani problematika HIV či dalších infekcí, které mohou ID způsobit.

Přesto, že kniha je zaměřena klinickým směrem včetně léčby, myslím, že svým systematickým uspořádáním a osvětlením patogenetických souvislostí může zaujmout i patology.

L. Peychl, Kolín