Molecular pathological examination of tight junction proteins and signaling pathways in fibrolamellar carcinoma

Attila Patonai MD

Ph.D. Thesis

Consultant: Prof. Dr. Zsuzsa Schaff M.D, Ph.D, D.Sc

Reviewers: Dr. István Tornai, med.habil., associate professor, Ph.D
Dr. Gábor Kovács med.habil., associate professor, Ph.D

Chairman of examination committee: Dr. Éva Keller, professor, MD, PhD

Members of examination comittee: Dr. László Herszényi associate professor, MD, Ph.D, DSc
Dr. Károly Simon Ph.D, head of Dept. of Pathology, Szent Imre Hospital

Budapest
2013

1.Background

Liver cancer ranks third in the case of men, and respectively fifth in the case of women as the main cause of cancer mortality in the world. Hepatocellular carcinoma (HCC) is the most common form of primary, malignant liver cancers that from a histological point of view can be divided into different subtypes. Chronic hepatotropic viral infections (hepatitis B virus – HBV; hepatitis C virus - HCV), chronic alcohol consumption, aflatoxin B1, the consumption of food contaminated with mycotoxin, fatty liver diseases (NASH/NAFLD), diabetes mellitus type 2, primary biliary cirrhosis, autoimmune hepatitis, hemochromatosis, Wilson's disease, as well as other inherited liver diseases, occupational diseases and many inborn metabolic disorders are among the factors that play a considerable role in the formation of HCC. Liver diseases, especially chronic hepatitis or liver cirrhosis, are responsible for about 70-90% of HCC incidence, while 10-30% of HCC cases are not related to cirrhosis.

Fibrolamellar carcinoma (fibrolamellar hepatocellular carcinoma, FLC) is a rare variant of HCC that typically affects young adults without underlying liver cirrhosis; its etiopathogenesis is unknown. FLC as a histologically distinct variant of conventional hepatocellular carcinoma (cHCC) was first described by Edmondson and his colleagues in 1956.

FLC incidence runs to about 1-5% of all HCC cases depending on geographical region under examination, however, no exact data are available in Hungary. According to literature the number of FLC cases represent 1-2% of all HCC incidence in the USA. In certain countries this rate is for unknown reasons higher, going up to 5.1% in Poland, and even 5.8% in Mexico. It is characteristic of FLC to express alpha-fetoprotein (AFP) and cytokeratin (CK)19 negative, as well as hepatocellular tumour markers (hepatocyte specific antigen - HSA, CK8, -18), and cholangiocellular markers (CK7). FLC patients do not have a considerably better outcome than cHCC ones, relatively favourable prognosis is due to the fact that FLC usually affects young adults with no underlying cirrhosis or fibrosis, with a high resecability rate. Possible FLC treatment may include tumour resection, liver transplant, interventional therapies and chemotherapy.

Epithelial and endothelial cells of multicellular organisms, are connected to each other by typical cell adhesion structures, of these tight junction (TJ) is to be found at the most apical pole of the cell. Tight junctions are built by protein complexes including occludin and claudins (CLDNs) as key components of the TJ structure. Besides these, tight junctions contain different proportion of junctional adhesion molecules (JAMs), as well as other proteins (cingulins, symplekins, ZO-1, E-cadherins, actin etc.). TJs have remarkably complex roles ranging from barrier formation through paracellular holes, to the regulation of ion- and liquid transport, and the establishment and maintenance of cell polarity. Recent research has proved that TJs take part in the regulation of numerous signal transduction processes, as well as in the establishment and maintenance of inborn immunity. The most important integral membrane proteins in TJs are occludin (OCLN) and claudins. Claudins can be divided into classic and non-classic claudins, and furthermore according to their function they can be tightening and channel

forming. Their composition and distribution in tissues can be highly different depending on the role played by the cells of the given tissue in paracellular ion transport and electric resistance. Claudin expression patterns show typical organ and tissue specificity, and so do tumours emerging from certain tissues.

Besides the above mentioned proteins two more recently discovered TJ associated Marvel proteins are known, tricellulin (TRIC) and MarvelD3 protein. Under physiological circumstances TRIC is mainly responsible for the regulation of the central part of tricellular tight junctions (tTJ) and bicellular tight junctions (bTJ).

Tyrosine kinases are tyrosine specific protein kinase enzymes that can only be found in multicellular organisms. Their highly important function is to transmit information related to increased expression of signals, adhesion, motility, and apoptosis between the cells of multicellular organisms. Some of the TKs are cell-surface (transmembrane) receptors, while others are to be found within the cytoplasm. They play a vital role in the regulation of growth, cellular differentiation, cell division, cell migration, proliferation and apoptosis. A considerable number of TK receptors are growth factor receptors (e.g. EGFR, PDGFR, IGF1R, SCF-ckit). The process of the regulation of signal transduction begins with the activation of the receptor at the moment of ligand binding during which, after the receptor-ligand binding, we witness dimerisation, followed by the downstream activation of further kinases (RAS-RAF, MEK-ERK, PI3K, AKT, STAT), phenomenon known as "downstream signalling". Tyrosine kinase inhibitors (TKI) bind to mutant protein, and thus have a tumour inhibiting effect. In case the KRAS gene is mutant no targeted anti-EGFR therapy can be used with the patient, as the presence of the abnormal KRAS protein obstructs the success of the treatment.

The β-catenin is a component of the adhesion junction, and its cadherin protein complex subtype connected to axin/conductin protein becomes a participant of central importance in intracellular signal modification in the Wnt/β-catenin signal transduction pathway. In the absence of active Wnt beta-catenin degrades and prospective target genes remain inactive. In case the Wnt signal is active β-catenin degradation decreases, and the thus accumulated β-catenin is able to activate transcription. Its important role has been shown in the case of head and neck squamous cell carcinoma, prostate carcinoma, colorectal carcinoma, and furthermore beta-catenin plays an important role in hepatocarcinogenesis.

Syndecan-1 (CD138) is a member of the heparin sulphate proteoglycan family and a marker of plasma cell differentiation. It has a considerable role in intercellular and cell-collagen connection, is also responsible for the regulation of the interaction between the cell and its extracellular environment, and impedes cell invasion into the collagen matrix. It regulates cell proliferation, cell migration, certain steps of signal transduction, and the structural variations of the cytoskeleton. It is expressed by numerous tumours, among which certain types of multiple myeloma, plasmacytoid cell tumours, diffuse large B-cell lymphoma, prostate, breast, colorectal, cervical, kidney, and head and neck squamous cell carcinoma.

2.Objectives

FLC is a rare variant of HCC, the etiopathogenesis of which is as yet unclear.

Our primary aim was therefore to explore molecular differences that help us make a distinction between FLC and other primary malignant liver tumours – first of all cHCC and cholangiocarcinoma (CCC) – while they help better characterise this tumour of relatively rare incidence.

Furthermore, we intended to present markers that besides enabling us to set up correct diagnosis, may become potential targets in antitumour therapy and help to understand better the pathogenesis of FLC.

We primarily felt bound to examine cell adhesion structures, mainly TJ proteins and certain signal transduction pathways, followed by mutations essential from a therapeutic point of view, such as EGFR and KRAS mutations.

In order to reach our aims we set ourselves the following tasks:

1. The examination of the expression profile of cell adhesion structure TJ proteins in FLC- claudins (1,2,3,4,5,7) and occludin – as compared to cHCC and CCC at protein and mRNS level;

2. The examination of the expression of recently recognised tricellulin (TRIC) in FLC, cHCC, and CCC primary liver tumours;

3. The examination of β-catenin and syndecan-1 protein expression in the case of FLC, cHCC, and CCC;

4. The examination of EGFR protein expression in FLC, cHCC, and CCC tumours;

5. The examination of EGFR and KRAS genes showing possible mutations in FLC.

3. Material and methods

3.1. Patients and Tissue Samples

We concluded our examinations with the authorization of the Regional Ethics Committee of Semmelweis University (#192) using cases of fibrolamellar carcinoma finalized with a diagnosis. Out of a total of 11 cases, 6 came from the material of the First Institute of Pathology and Experimental Cancer Research and Second Institute of Pathology of Semmelweis University archived between 1999 and 2009, 3 samples were sent from the provincial centres of Hungary (Gyöngyös, Szeged, Debrecen), and a further two examined cases were samples from the Pathology Institute of Ruprecht-Karls University from Heidelberg, Germany. Out of the 11patient material we examined 8 surgical resections, and 3 percutaneous liver biopsies, none of these cases showing liver cirrhosis or any virus infection. Out of the 11 patients 6 had been clinically proved to have had metastasis (lymphatic gland, lungs, peritoneum). In all of these cases diagnosis was set up on the basis of clinical symptoms, imaging techniques (ultrasound, CT, MRI), and histological examination.

The age of the patients was between 11 and 66 years (giving an average of 20.8 years), while the proportion of men/women was 5/6. All the FLC samples were compared to 7 conventional hepatocellular carcinomas (cHCC, grades 2-3), 7 cholangiocellular carcinomas (intrahepatic CCC, grades 2-3), and 5 non-tumorous control liver samples. Out of cHCC patients two cases showed virus infection, 3 cases proved to have had liver cirrhosis, while one patient had multiple tumour alteration. Choosing samples from patients of the same age group was not possible because according to data from literature the average age of FLC patients (20.81 years) is considerably lower than that of cHCC (66 years) and CCC (60 years) groups.

3.2. Histological examinations

Sample tissues were fixed in 10% neutral (pH 7.4) buffered formalin at room temperature for 24 hours, then were paraffin embedded after having been dehydrated in alcohol series and xylol. We then made 3-4 μm sections of the paraffin block embedded samples (FFPE), which were later stained with haematoxylin and eosin (HE) and pycrosyrius red for detection of fibrous tissue.

3.4. Immunofluorescence examinations

In order to be able to predict exact protein localization of TRIC, OCLN, CD31 immunofluorescence examinations were performed with primary antibodies. Samples were fixed in liquid nitrogen and isopentane. Frozen sections were incubated with antibodies against tricellulin (TRIC), occludin (OCLN), CD31, and fluorescence Alexa Fluor 488 (FITC) and/or Alexa 568 labelled secondary antibodies. We examined and photographed the frozen and labelled sections with a Leica DMRXA fluorescent microscope equipped with a Pieper FK-7512-IQ CCD camera that generates the given maximum range of fluorescent light. Then, we fixed the images with the help of Leica CW4000 FISH visualisation and documentation Software. The frozen sections were examined by using BioRad Radiance 2100 laser scanning confocal microscope and LaserSharp 2000 software.

3.5. Digital Morphometric analysis

We assessed immunohistochemical reactions in CLDN 1,2,3,4,5,7, TRIC, EGFR, syndecan-1, and β-catenin using the semiquantitative and the digital morphometric method. We digitalised sections using Mirax Panoramic MIDI and Mirax Panoramic SCAN digital slide scanners, measured and assessed the intensity and extension of different immunohistochemical signals as shown on digital images using Leica QWin Software.

3.6. Statistical analysis

We performed statistical analysis with the help of the non-parametric Kruskal-Wallis test using STATISTICA Software 8.0 version (Tulsa, OK, USA). When assessing results we considered an alienation of $P< 0.05$ as being statistically significant.

3.7. PCR examinations

3.7.1. Extracting RNA from tissue samples, cDNA synthesis, primary planning

In order to establish protein regulation levels we performed mRNA expression examinations. We isolated the complete RNA from FFPE materials. During this process we placed 8-10 3-5µm thick slices of paraffin sections from the paraffin blocks of the FLC samples into 1.5 ml sterile plastic tubes, then we isolated RNA from these, using the QIAGEN RNeasy FFPE Kit (Cat.No.: 73504, QIAGEN GmbH, Hilden, Germany) upon the recommendation of the producer, and also following their instructions for use. We checked the purity and concentration

of the resulting nucleic acid through OD measurement using NanoDrop 1000 Spectrophotometer (ThermoFisher Scientific Inc., Wilmington, DE, USA) device. Reverse mRNA transcription into cDNA was performed using a total of 1µg of RNA per sample, and the High Capacity RNA-to-cDNA Kit (Applied Biosystems/Life Technologies, Carlsbad, CA, USA). We designed primary form specific genes using AlleleID 6.01 (Premier Biosoft International, Palo Alto, CA, USA) primary design software. Isoform specificity and the sizes of primaries were determined with the help of BioEdit („biological sequence alignment") editing software 7.01 (Tom Hall Ibis Therapeutics, Carlsbad, CA, USA). Specificity of given primaries was checked with the help of BiSearch software (Institute of Enzymology, Budapest, Hungary).

3.7.2. qPCR

The quantity of mRNA of the sample/product as compared to the housekeeping gene was determined with real-time PCR examination using synthesized cDNA. In order to examine the quantity of mRNA determining the target protein we performed real-time polymerase chain reaction (PCR, qPCR) working with SYBR Green technology on an ABI Prism 7000 Sequence Detection System (Applied Biosystems). As housekeeping genes ABL (BCR-ABL oncogen) and 36b4 (ARP, human acidic ribosomal phosphoprotein-PO) were used. Primary specific amplification was assessed using melting analysis and 2% agarose gel electrophoresis. In order to statistically assess data in the course of qPCR examinations REST software (Relative Expresssion Sofware Tool; www.wzw.tum.de/gene-quantification) was used.

3.8 Examination of KRAS mutation with Microfluidic Capillary Electrophoresis-Based Restriction Fragment Length Polymorphism Method (RFMD)

In order to isolate DNA, 4 3-5µm thick samples were resected out of FFPE tissue samples under sterile conditions, and were later transfered into 1.7 ml plastic tubes (Eppendorf AG). We isolated DNA with the help of QIAmp®DNA FFPE Tissue Kit (QIAGEN) following the instructions of the producer. The quantity of the obtained DNA was determined through absorption spectrum examination and OD measured at 260 nm wavelength with a spectrophotometer (ND-1000, Thermo Fisher Scientific). DNA was amplified using AmpliTaq Gold (Applied Biosystems) polymerase enzyme, and Mastercycler® thermal cycler (Eppendorf AG) device. The composition of the reaction mixture by reaction and by sample was the following: 2.5 µl 10x PCR puffer + $Mg2^{+}$, 200 µM/ dNTP, 1pM/reaction for every single primary, using 0.8 U AmpliTaq Gold DNS polymerase sense mismatch primary the PCR product

contained the wild type KRAS gene BstNI or the Bg1I restriction endonuclease recognition sequence. Each reaction was completed with 38 cycles respecting the following conditions: denaturation at 95°C for 1 minute, primary annealing at 55°C for 1 minute, chain elongation at 72°C for 2 minutes. The amplified product was digested with 80U BstNI for the KRAS codon 12 mutation, and with 80U BglI for the KRAS codon 13 mutation (New England BioLabs). Enzymatic digestion was set at 30 µl total volume at 60° in the case of KRAS codon 12, and at 37°C in the case of KRAS codon 13. Digested PCR product was examined following agarose gel ethidium bromide staining with microfluidic-based Experion (BioRad Laboratories Inc.) gel electrophoresis system using BioRad Experion™ DNA 1K Analysis Kit.

3.9. EGFR mutation examination

EGFR exon 18, 19, 20, 21 amplicons were examined using High Resolution Melting procedure and Roche Light Cycler 480 Real Time PCR (Roche Diagnostics) device. Evaluation was done using the Gene scanning programme inbuilt into the device.

3.10 Direct sequencing

The results of High Resolution Melt Analysis were validated by direct sequencing. Following the examinations of exons 18 and 21, taking into consideration the fact that results were clearly wild type, direct sequencing was performed, and also exons 19 and 2 were validated using the same independent method. The reaction was performed using BigDye® Terminator v1.1 cycle Sequencing Kit (Applied Biosystem) respecting the instructions of the manufacturer. The sequencing reaction was analysed using ABI 3130 Genetic Analyser System (Applied Biosystems) device after having purified it using BigDye® X terminator purification kit (Applied Biosystems) and it having undergone sequencing PCR.

4. Results

4.1. Macroscopy of FLC

The typical macroscopic appearance of FLC was to be seen in 8 surgical resections out of the 11 examined cases. In all removed specimens the resected area of the tumour was soft to the touch, yellowish-white in colour, with central or excentric scars of different thickness and sometimes bifurcating on the septally structured connective tissue.

The average diameter of tumours was found to be 86x73x76 mms, liver tissue architecture around the tumour was in every case normal, no fibrosis or cirrhosis could be detected.

4.2. Histological characteristics of FLC

All 11 FLC cases presented a typical histopathological image. The carcinoma is made up of tumour cells of polygonal shape containing eosinophilic cytoplasm, and which organise into columns or networks sporadically creating glandular structures. Groups of tumour cells were divided by fibrotic septum having typical “lamellar” structure.

4.3. Immunohistochemistry, immunofluorescence, and morphometric analysis

Immunohistochemical examinations in FLC brought characteristic results. CK7, CK8, and CK18, as well as HSA (HepPar1) were positive, nevertheless, AFP and CK19 were negative in all FLC samples examined.

Glypican-3 (GPC3) gave a negative result in the greatest majority of cases, weak, focal cytoplasmic reaction in tumour cells was found in only two cases out of eleven.

CLDN1 and CLDN2 presented similar pattern in all three groups of tumour examined. Immunohistochemical reaction performed with anti-CLDN1 antibody appeared in intensive, membranous, typical “honeycomb” structure completely surrounding the tumour cells. On the basis of morphometric examinations, significantly higher CLDN1 expression could be detected in the case of CCC as compared to control groups, taking them in order, in cHCC and FLC, and respectively the lowest CLDN1 expression was shown in normal liver tissue. From the point of view of CLDN1 expression the difference between the FLC and cHCC groups proved to be significant. CLDN2 expression in FLC was definitely strong, immune reaction gave granular cytoplasmic/submembranous type positivity on tumour cells, nevertheless, this difference was not significant as compared to normal liver.

In the case of CLDN4 and CLDN7 immunohistochemical reactions in cHCC and

FLC tumour groups proved negative, by contrast with CCC, where strong, intensive membranous positive reaction could be seen in the case of CLDN4. The CLDN4 protein expression is significantly higher in CCC according to immunohistochemical and morphometric examinations than in other groups examined in which it practically proved negative.

CLDN5 proved to be positive with variable intensity in 9 out of 11 FLC cases examined, by contrast with cHCC, CCC and normal control liver in which it was negative. According to statistical examinations performed on the basis of data obtained through morphometric examinations, CLDN5 protein expression was significantly higher as compared to normal liver. CLDN5 expression in FLC was also considerably higher than in cHCC.

In the course of immunohistochemical examinations of tricellulin (TRIC) and occludin (OCLN) in FLC reaction presented itself in the form of linear and locally dot-like membranous positivity corresponding to pseudoglandular structures, and alongside tumour cells respectively. In normal liver TRIC and OCLN showed dot-like immune response in the case of two and three cell-cell adhesion, while at the apical pole of hepatocytes, and on biliary epithelial cells intensive reaction could be detected. TRIC protein expression was higher in well-differentiated trabecular or pseudoglandular parts of the tumours, while it was considerably lower or almost absent in their solid or anaplastic parts.

Immunohistochemical examination of EGFR proved intensive positivity associated to the plasma membrane. As compared to normal liver EGFR protein expression was higher in all tumours examined.

β-catenin immune response showed membranous positivity in all cancerous tissues examined. Nuclear translocation/ nuclear positivity seen were evident neither in FLC, nor in cHCC and CCC samples.

Syndecan-1 (CD138) immunohistochemical examination showed membranous/submembranous positivity in all tumour groups examined. More intensive positivity was to be seen in the case of FLC and cHCC as compared to CCC. The most widespread positivity was detected in cHCC, nevertheless, intensive membranous positivity was detected in FLC as well.

4.4. mRNA expression analysis

In the course of mRNA expression examinations we assessed relative expression as compared to reference genes. We found mRNA expression of CLDN1 and TRIC in the cHCC group as being higher as compared to FLC and normal liver groups. By comparison, mRNA

expression of CLDN1 and TRIC in normal liver was lower. mRNA expression of CLDN1 and TRIC was found to be the lowest in the case of FLC. In the case of CLDN2 the mRNA expression of the normal liver was the highest, followed by the cHCC, and then by the FLC groups. In the case of CLDN4 and CLDN5 by far the highest mRNA expression was given by normal liver, followed by FLC, and then cHCC groups. None of the above mentioned differences in mRNA expression proved to be statistically significant.

4.5. KRAS and EGFR mutation analysis

In the area of codons 12 and 13 on exon 2 of the K-RAS gene in examined FLC samples no mutation was found.

Using high resolution melt technique (HRM) and direct sequencing no mutation could be detected in exons 18, 19, 20, and 21 of the EGFR gene.

5.Conclusions

Tight junction proteins (TJ proteins) present a characteristic, typical pattern in FLC. The pattern of claudins in FLC is similar to cHCC, and is different from the typical image of CCC.

We were the first to show that FLC expresses to a considerable extent CLDN5 that is primarily typical of endothelial cells, and this is not detectable in other primary liver carcinomas.

We were the first to prove the expression of tricellulin as a recently described TJ protein in FLC.

We showed increased EGFR expression in FLC, which however, does not imply gene mutation, and no mutation of the KRAS gene was proved.

According to the above, FLC is a special subtype of HCCs that can be clearly distinguished from other primary liver cancers on the basis of its TJ protein pattern.

The proved increased CLDN5 and EGFR expression can become the target of future therapies.

6. New Results of the Thesis

1. We were the first to show typical changes of TJ protein patterns in FLC.

2. We showed increased CLDN5 expression in FLC as opposed to other primary liver tumours, and this can become the aim of future therapies.

3. We demonstrated that CLDN4 negativity in FLC tumour proves its similarity to cHCC, and distinguishes it from CCC.

4. We were the first to show tricellulin expression in FLC and its nuclear positivity.

5. We proved increased EGFR expression in FLC, and also the absence of EGFR and KRAS gene activating mutations in FLC. All this can potentially become the basis of further therapeutic considerations.

6. Considering the above, we can state that FLC is a special subtype of HCC that can be clearly distinguished from other primary liver cancers on the basis of its TJ protein pattern.

7. The proved increased CLDN5 and EGFR expression can become the target of future therapies in FLC.

7.LIST OF PUBLICATION

Publications related to the thesis ;

Patonai A, Erdélyi-Belle B, Korompay A, Somorácz Á, Straub BK, Schirmacher P, Kovalszky I, Lotz G, Kiss A, Schaff Zs.
Claudins and tricellulin in fibrolamellar hepatocellular carcinoma
Virchows Archiv - An International Journal of Pathology 458:(6) pp. 679-688. (2011)
IF: 2.491

Patonai A, Erdelyi-Belle B, Korompay A, Somoracz A, Torzsok P, Kovalszky I, Barbai T, Raso E, Lotz G, Schaff Zs, Kiss A
Molecular Characteristics of Fibrolamellar Hepatocellular Carcinoma.
Pathology & Oncology Research 19:(1) pp. 63-70. (2013)
IF: 1.366

Publications non-related to the thesis ;

Csébi Péter, Németh Tibor, Jakab Csaba, Patonai Attila, Garamvölgyi Rita, Manczur Ferenc, Spitzner A, Arany Tóth Attila, Kóbori László
Experimental results of using autologous rectus fascia sheath for venous patch grafts in dogs
Acta Veterinaria Hungarica 59:(3) pp. 373-384. (2011)
IF: 0.673

Jakab Cs, Csébi P, Szász AM, Szabó Z, Patonai A
Krónikus, óriásredős gyomorgyulladás kutyában: Chronic hypertrophic gastritis in a dog. Case report
Magyar Állatorvosok Lapja 131: pp. 601-609. (2009)
IF: 0.200

Kobori L, Nemeth T, Nagy P, Dallos G, Sotonyi P, Fehervari I, Nemes B, Gorog D, Patonai A, Monostory K, Doros A, Sarvary E, Fazakas J, Gerlei Z, Benko T, Piros L, Jaray J, De Jong KP
Experimental results and clinical impact of using autologous rectus fascia sheath for vascular replacement

Acta Veterinaria Hungarica 56:(3) pp. 411-420. (2008)
IF: 0.624

Horvath A, Patonai A, Banhegyi D, Szlavik J, Balazs G, Gorog D, Werling K
[The first case of human alveolar Echinococcosis in Hungary]; A human Echinococcus multilocularis infectio első hazai esete.
Orvosi Hetilap 149:(17) pp. 795-799. (2008)

Nemes B, Sárváry E, Kóbori L, Gerlei Zs, Patonai A, Perner F, Weszelits V, Járay J, Schaff Zs
Serum hepatitis C virus-ribonucleotide acid monitoring after liver transplantation
Digestive and Liver Disease 37:(1) pp. 68-69. (2005)

Lengyel G, Kóbori L, Fehérvári I, Nemes B, Görög D, Patonai A, Sárváry E, Varga M, Hagymási K, Perner F, Fehér J, Tulassay Zs
Combined interferon-alpha-2b and ribavirin therapy in patients with recurring chronic hepatitis C (genotype 1) following liver transplantation in Hungary
Archives of Medical Science 1:(1) pp. 8-12. (2005)

Lotz G, Simon S, Patonai A, Sótonyi P, Nemes B, Sergi C, Glasz T, Füle T, Nashan B, Schaff Zs
Detection of Chlamydia pneumoniae in liver transplant patients with chronic allograft rejection
Transplantation 77:(10) pp. 1522-1528. (2004)
IF: 3.568

Lengyel G, Kóbori L, Fehérvári I, Nemes B, Görög D, Patonai A, Sárváry E, Varga M, Perner F, Fehér J
Kombinált interferon-alfa-2b és ribavirin terápia májtranszplantációt követő krónikus C-hepatitisben
Orvosi Hetilap 144:(48) pp. 2367-2370. (2003)

Patonai A, Nemes B, Görög D, Kóbori L, Sótonyi P Jr, Fehérvári I, Weszelits V, Doros A, Dallos G, Schaff Z, Perner F
A hazai májtranszplantációk értékelése patológiai szempontból [Pathologic evaluation of orthotopic liver transplantation in Hungary]

Orvosi Hetilap 142:(9) pp. 435-441. (2001)

Patonai A, Csikós A, Deák G
Visceral aluminum deposition in chronic renal insufficiency - Light microscopy and X-ray microanalysis
Pathology & Oncology Research 2:(1-2) pp. 94-97. (1996)

Abstracts related to the thesis ;

Somoracz, A., Korompay, A., Patonai, A., Erdelyi-Belle, B., Toerzsoek, P., Lotz, G., Schaff, Z., Kiss, A.
Expression of tricellulin, a recently identified tight junction protein in normal liver and in primary liver cancers
Journal of Hepatology, Volume: 54, Mar, 2011, ISSN: 0168-8278
46th Annual Meeting of the European Association for the Study of the Liver (EASL)
2011, Berlin, Germany

Somoracz A., Korompay A., Toerzsoek P., Patonai A. Erdelyi-Belle B., Lotz, G., Schaff, Z. , Kiss, A.
Tricellulin expression correlates with overall survival in hepatocellular and cholangiocellular carcinomas
Journal of Hepatology Volume: 56 Apr.,2012. ISSN: 0168-8278 Accession Number:
47th Annual Meeting of the European Association for the Study of the Liver (EASL)
Apr. 18-22, 2012, Barcelona, Spain

Abstracts non-related to the thesis ;

Z. Schaff, G.Lotz, A.Patonai, Á. Szepesi
Cyclin A and PCNA expression in chronic hepatitis C; J Hepatol, 26, 1997, p.144

Á. Gohér, K.Bach, L.Szalay, A.Patonai, Zs.Schaff, F.Perner, Zs.Tulassay
Liver transplantation in the therapy of metastases of an undifferentiated mesenchymal tumour Z Gastroenterol,1996, Suppl.1, 5.

Schaff Zs, Jármay K, Szepesi Á, Szalay F, Patonai A, Nemes B, Lotz G, Lonovics J
Correlation of transforming growth factor beta-1, apoptosis and proliferation in chronic hepatitis; J Hepatol., 28 1998, Suppl. 1: 101

G.Lotz, P.Nagy, A.Patonai, A.Kiss, B.Nemes, F.Szalay, Zs.Schaff
TGFβ-1 and apoptosis in human hepatocellular carcinoma and focal nodular hyperplasia
J Hepatol, 28, 1998, Suppl.1 :175

G.Lotz, P.Nagy, A.Patonai, A.Kiss, B.Nemes, F.Szalay and Zs.Schaff
Hepatocyte proliferative index correlates with the risk of developing HCC in cirrhosis
J Hepatol, 28, 1998, Suppl. 1 co4/003

A.Patonai, B.Járay, J.Pápay, E.Székely, T.Winternitz, B.Nemes, A. Doros, L.Berczi, Zs.Schaff
US controlled fine needle aspiration biopsy (FNAB) in diagnosis of cystic hepatic echinococcosis; Acta Cytol. 1999 July-Aug. No.4.,vol.43, p.740

Zs.Schaff, Á.Szepesi, A.Patonai, K.Jármay
Detection of apoptosis and proliferation in chronic hepatitis c before and after interferon treatment; J Hepatol. 30 1999, Suppl. 1:124

A.Patonai, G.Kovács, B.Erôss, B.Nemes, G.Lotz, Zs.Schaff, F.Perner
Acute rejection in liver transplantation; correlation of laboratory parameters with histopathological findings; Z Gastroenterol, 1998, 5., 36, p: 440

G.Lotz, B.Nemes, A.Patonai, Zs.Simon, A.Mohos, Z.Schaff
Detection of HCV-RNA by RT-in situ PCR after liver transplantation
J Hepatol.30 1999, Suppl. 1:173

A.Patonai, B.Nemes, D.Görög, F.Perner, Zs.Schaff
Liver transplantation for benign and malignant tumours
J Hepatol. 30 1999, Supplement 1:182

Bögi K, Patonai A, Kiss A, Erőss B, Kovács G, Nemes B, Schaff Zs, Perner F
Acute rejection in liver transplantation, correlation of laboratory parameters with histopathological findings; J. Hepatol. 30, 1999, Suppl. 1: 181

Schaff Z., Lotz G., Simon S., Patonai A., Sótonyi P., Nemes B., Georgopoulos A., Graninger W.
Detection of chlamydia pneumoniae in liver transplant patients with chronic allograft rejection; J Hepatol, 34, Suppl. 1, April 2001, pp. 43 (1)

Schaff Zs, Lotz G, Simon S, Patonai A, Sótonyi P, Nemes B, Georgopoulos A, Graninger W
Detection of Chlamydia pneumoniae in liver transplant patients with chronic allograft rejection
Z. Gastroenterol. 2001 39: 5 May

Patonai A, Nemes B, Görög D, Kóbori L, Sótonyi P, Fehérvári I, Weszelits V, Doros A, Dallos G, Schaff Zs, Perner F.
Pathological evaluation of different complications of liver transplantation in Hungary - 5 years experience; Z. Gastroenterol. 2001 39: 5 May

Patonai A, Nemes B, Sárváry E, Kóbori L, Lengyel G, Weszelits V, Fehérvári I, Perner F, Schaff Zs.
Liver transplantation due to HCV - The problem of recurrence; J Hepatol 34 Suppl. 1, April 2001 pp.35

Gonda P, Patonai A, Szönyi L, Schaff Zs.,Gyermekkori májbetegségek a biopsziás vizsgálatok tükrében, Z. Gastroenterol. 2001. 39: 5 Mai

Patonai A, Kóbori L, Tóth Á, Nagy K, Kiss A, Schaff Zs; Fulminant visceral Kaposi's sarcoma after orthotopic liver transplantation, Pathologica 94: 82, 2002

Gonda P, Patonai A, Szönyi L, Kiss A, Schaff Zs; Childhood liver biopsy; Pathologica 94: 80, 2002

G.Lotz G, Patonai A, Simon S, Sergi C, Nashan B, Schaff Zs ; Localization of Chlamydia pneumoniae by immuno-histochemistry and in situ PCR in chronic liver allograft rejection with obliterative arteriopathy; J Hepatol , 36 Supplement 1, 2002, pp. 40

Book section:

Perner F., Petrányi Gy.; Szervátültetés (Organ transplantation). Schaff Zs., Patonai A., Nagy P.; A májtranszplantáció patológiája. (Pathology of liver transplantation) Medicina Könyvkiadó Zrt., 2013