Uncovering some ignored pioneering works on post-infectious irritable bowel syndrome

To the Editor,

Post-infectious irritable bowel syndrome (PI-IBS) is a recently described subtype of IBS [1] which has become officially accepted in the Rome III classification [2]. This term describes those cases of IBS which occur after intestinal infections [3], and the pathogenesis of the symptoms is produced, as shown on a large sample, by genetically influenced intestinal wall inflammation triggered by the infection [4]. But it seems that the first reporting of the medical condition now called PI-IBS is actually older.

We present here data from a series of papers published almost 50 years ago by the group led by Octavian Fodor, at that time Head of the 3rd Medical Department (focus on gastroenterology) in Cluj, Romania. These investigators are precursors of the PI-IBS research. Their results were published in French and German, not in English, and this is maybe the reason why these pioneer works have not been credited worldwide.

Using blind small bowel biopsies in patients with chronic abdominal symptoms: pain, diarrhea, bloating, the authors observed inflammatory infiltrates in the absence of any significant organic condition or of a specific inflammation, similar to IBD. They coined the term chronic nonspecific enteropathy [5]. The authors described the general frame of bowel disorders and associated the small bowel symptoms with a more general disorder, including the colon. The condition would now be defined as IBS-D or IBS-M (diarrhea- or mixed-type IBS). This study detected also inflammation in the jejunal mucosa of 29.8% of the patients with chronic nonspecific enteropathy. A few years later the authors published a continuation of their work, this time with the emphasis on morphological changes of the bowel mucosa [6]. In this paper, the investigators insisted on the nonspecific character of the condition and emphasized the presence of mucosal inflammation not related to any other specific disease. Inflammation in IBS is now largely accepted [4].

A third paper was issued a few years later, describing the immune mechanisms of inflammation of the small bowel mucosa in the context of the chronic nonspecific enteropathy [7]. The authors differentiated the chronic nonspecific enteropathy from ulcerative colitis.

Over the following years the authors reported the connection between acute infectious enterocolitis and the chronicization of symptoms, in chronic nonspecific enteropathy [8]. In this study, 43.6% of patients with chronic nonspecific enteropathy from a sample of 540 patients recognized at least one episode of acute gastrointestinal infection in their history. We reproduce in Fig. 1 the histological transition from infectious enterocolitis to chronic nonspecific enteropathy [from reference 8].

Thus, in a timeframe of 10 years, several papers from this group in Cluj were able to identify a new pathological condition characterized by nonspecific abdominal complaints, different degrees of inflammation in the intestinal mucosa and in almost half of the cases, a history of acute enteritis. This condition overlaps very much IBS-D, and the cases starting after acute intestinal infections are similar to the cases that we diagnose now as PI-IBS.

Stewart described even before a chronic condition called post-dysenteric colitis [9], becoming a precursor of PI-IBS description. But the works by Fodor et al refer to cases triggered by nonspecific, not dysenteric intestinal infections.

The history of medicine shows frequently cases where a new term, disease, therapy etc. has been actually observed or described in advance by others, who were not able to impose their priority. This is also the case of this group who described a new disease: chronic nonspecific enteropathy, totally superposable to the present condition called PI-IBS.

Despite the little attention to these studies at that time, this entity can now be perceived as probably the true first description of PI-IBS.

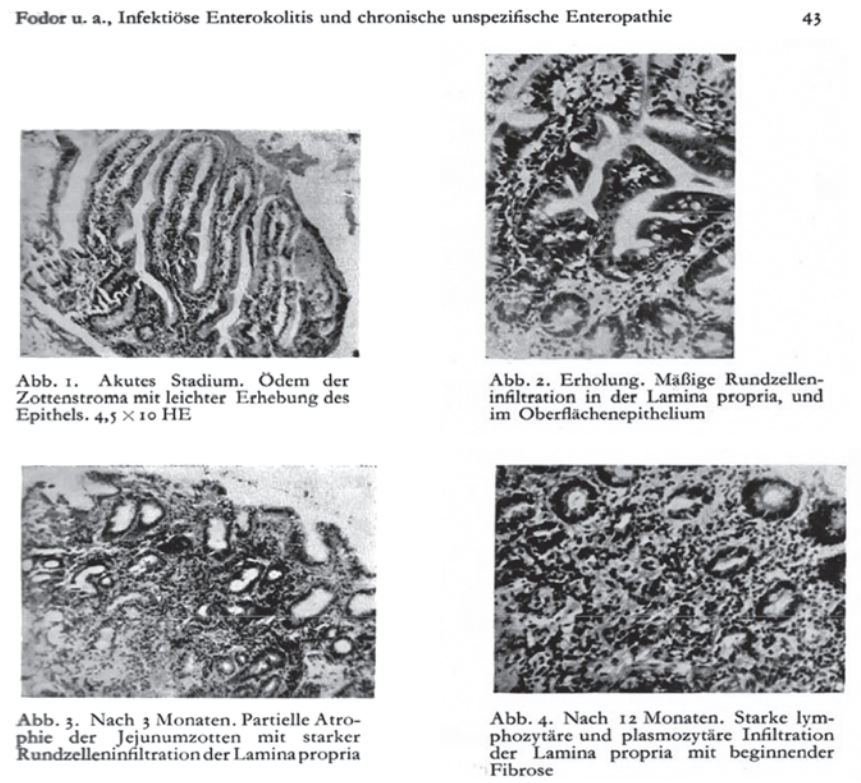

Fig. 1 (ref. 8). Histological aspect of jejunal mucosa after infectious enterocolitis: evolution in time with the persistence of inflammation 12 months after the acute episode. 1: acute inflammation; 2: recovery stage: round cell infiltrate; 3: after 3 months: partial mucosal atrophy and round cell infiltrate; 4: after 12 months: lymphocytes and plasmocytes in lamina propria.

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Hepatitis C virus infection in end stage renal disease and after kidney transplant

To the Editor,

Hepatitis C virus (HCV) infection remains the main cause of liver disease in candidates for kidney transplantation (KT) [1–3]. Determining the severity of liver disease is often difficult since transaminases may be persistently normal even in the presence of important liver damage [4–6] and also due to the fact that liver biopsy is generally avoided given the increased bleeding risk. The aim of the present study was to evaluate the impact of HCV in this population and in patients who undergo KT, considering the threat of hepatitis flares, especially with immunosuppression, and the fact that antiviral therapy can be challenging due to anemia of chronic renal disease.

All anti-HCV positive KT candidates who were prospectively and consecutively seen at the Hepatology outpatient clinic from January 1995 to January 2010 were studied and followed until the closure of the study. Subjects with concomitant HBV hepatitis and/or HIV infection were excluded. Fifty-four patients were anti-HCV+. HCV-RNA was positive in 38/54

(70.4%) patients, and the mean value was 3,723,940.34 ± 7,550,525.83 copies/ml. The genotype was available in 21 out of the 38 HCV-RNA positive patients, and 11 of these (52.3%) had HCV-genotype 1b infection. No clinical, endoscopic, or ultrasound signs of portal hypertension were identified in any of the 54 patients. Mean follow-up was 172.4 months (±24.4 months), and median follow-up was 180 months. Liver biopsy was performed in 29 patients, demonstrating fibrosis F0 in 10, F1 in 10, F2 in 7, and F≥3 in 2 patients (Metavir scoring) [7]. According to the virological and histological findings, a local scoring system to assess the risk of progression of liver disease after KT was created (Table I). The local score for predicting the likelihood of liver disease progression showed a low risk in 4, low/intermediate risk in 7, intermediate in 12, intermediate/high in 4, and high risk in 2 patients.

Eight patients were treated with interferon 3 mil IU three times per week for six months, obtaining sustained virological response in three individuals. Thirty-eight patients received a KT, and standard immunosuppression was used in all. Of the 21 KT recipients, 6 had undergone antiviral therapy, and 3 had achieved SVR and were HCV-RNA negative at the time of KT. During follow-up, no episodes of hepatitis were detected in KT recipients.

Table I. Local risk evaluation system for the prediction of liver disease progression in HCV positive ESRD patients awaiting KT

Risk evaluation	HCV RNA status	Genotype	Histology/Clinical, endoscopic, and radiological criteria*
Low	Negative	-	No portal hypertension
Low/intermediate	Negative/ Positive	- / non-1b	G=1-2; S=0-1
Intermediate	Positive	1b	G=1-2; S=2
Intermediate/high	Positive	1b	G=2-3; S=2
High	Positive	1b	G=3; S=3-4

ESRD: end stage renal disease; G: grading; HCV: Hepatitis C Virus; KT: kidney transplantation; S: staging. *Clinical, endoscopic, and radiological criteria determined the absence of portal hypertension in all studied patients

Patient survival was 100% at 1 year and 96.4% at 5 years after KT, similar to reported survival rates in the overall adult KT recipient population at our center (98.6% and 92.6%, at 1 and at 5 years after KT, respectively, p=0.92), and overall in Italy (97.1% and 92.1%, at 1 and at 5 years post KT, respectively, p=0.88) (Table II) [8]. Long-term patient survival in the present series was 92.1% and 76.3% at 10 and 15 years post KT. Death-censored graft survival was 95.7% at 1 year, 84.9% at 5 years, and 70% at 10 years after KT, comparable to data reported regarding overall 1-year (95.7% at our center and 91.9% overall in Italy, p=0.98) and 5-year (84.9% at our center and 81.7%, overall in Italy, p=0.97) graft survival after adult KT. All deaths were unrelated to hepatic disease: renal cancer recurrence (1 patient), intestinal perforation (1), dialysis complications (2) and cardiovascular disease (5 patients).

In conclusion, the long follow up (up to 15 years after transplant) showed survival rates similar to those of non-HCV patients. The use of a scoring system including histological features and viremia effectively allows for patient stratification regarding antiviral therapy and prognosis of liver disease.

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Table II. One- and five-year patient and graft survival after kidney transplant

No of patients who survived / total no transplanted	Patient survival 1 year post KT	Graft survival 1 year post KT	Patient survival 5 years post KT	Graft survival 5 years post KT
Present study	100% (38/38)	97.3% (37/38)	96.4% (28/29)	96.4% (28/29)
Overall at local center [8]	98.6% (437/443)	95.7% (424/443)	92.6% (NA)	84.9% (NA)
Overall in Italy [8]	97.1% (12,921/13,307)	91.9% (12,229/13,307)	92.1% (NA)	81.7% (NA)

KT: kidney transplantation; NA: data not available.

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Tenofovir-associated Fanconi syndrome in a patient with chronic hepatitis B

To the Editor,

Fanconi syndrome is an uncommon adverse reaction of tenofovir (TDF) treatment in HIV-infected patients. However, it has rarely been reported in patients with chronic HBV monoinfection: there is only one such report in the literature [1].

An 82-year-old man with type 2 diabetes mellitus, Parkinson's disease and liver cirrhosis due to hepatitis B virus (HBV) infection was admitted because of a lower respiratory tract infection. He had been receiving lamivudine and adefovir up to six months before his admission, when he was switched to TDF monotherapy, in accordance with the 2012 EASL guidelines [2].

Arterial blood gases measurement revealed a normal anion gap metabolic acidosis with pH 7.37 and bicarbonate 13.9 mEq/L. Intravenous bicarbonate loading resulted in the increase of blood pH to 7.45, of urine pH from 6.0 to 8.0, and of serum bicarbonate level to 18 mEq/L thus confirming the presence of type II renal tubular acidosis (RTA). There was also hypophosphatemia (1.1 mg/dL), hypouricemia (1.7 mg/dL), glycosuria and mild proteinuria suggesting Fanconi syndrome. This was diagnosed by documenting renal phosphate wasting: 24 h urine phosphate was 430 mg and the fractional excretion of phosphate was 65.8%.

Causes of Fanconi syndrome such as monoclonal protein disorders, hyperparathyroidism, vitamin D deficiency and heavy metal intoxication were excluded by history, clinical findings or appropriate investigations. Of the drugs known to be associated with Fanconi syndrome, the patient was receiving TDF; therefore, we diagnosed a TDF–associated Fanconi syndrome. Oral bicarbonate supplementation was initiated and TDF was discontinued with a plan to start entecavir. However, two months later the patient, with glycosuria, proteinuria, hyphosphatemia and hypouricemia still present, died of severe sepsis.

Fanconi syndrome is a proximal renal tubular dysfunction characterized by impaired reabsorption of bicarbonate, phosphate, glucose, uric acid and amino acids leading to normal anion gap acidosis, hypophosphatemia, renal glycosuria, hypouricemia and/or aminoaciduria. Clinical manifestations in adults include polyuria, polydipsia, dehydration, and osteomalacia. The causes of the syndrome in adults are drugs and toxins, heavy metals, monoclonal protein disorders, vitamin D deficiency and disorders such as Wilson's disease and paroxysmal nocturnal hemoglobinuria [3].

Tenofovir, a nucleotide reverse transcriptase inhibitor, is among the preferred first line medications for the treatment of HBV infected patients [2]. Fanconi syndrome in association with TDF is rare and it has been reported almost exclusively in patients with HIV or HIV-HBV co-infection [4]. The existing data from patients with HBV monoinfection suggest that TDF nephrotoxicity and Fanconi syndrome might occur less frequently than in HIV patients [5, 6]. This difference could be due to the HIV-induced nephropathy per se and to the reduced renal clearance of TDF when combined with certain antiretrovirals for the treatment of HIV infection [7]. However, there might be a reporting bias, as physicians caring for HIV infected patients may be more aware of TDF nephrotoxicity.

Although Fanconi syndrome is a rare complication of TDF treatment, it has long term consequences, and therefore physicians caring for HBV-infected patients should be able to detect it early.

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Clostridium difficile is emerging in Romania: a story of 027 ribotype and excessive antibiotic consumption

To the Editor,

We read with real interest the paper by Lupse et al [1] and the subsequent comments [2, 3]. Indeed, the emergence of *Clostridium difficile* infection (CDI) is a hot topic for the Romanian healthcare system, and each new paper on this topic is valuable for us, as are the papers describing CDI in other areas of the world.

We agree with the analysis performed by the authors concerning the risk factors for the recurrence of CDI. However, we suggest that the administration of a systemic antibiotic after

the onset of CDI requires also to be analyzed, because it has a greater importance for recurrence than the previous treatment with antibiotics, as indicated by Mullane et al [4].

We also agree with our colleagues' statement about the variability of clinical manifestations of CDI [3]. Watery diarrhea could be an indicative symptom for CDI, but a fast diagnosis of severe CDI cases without diarrhea is more important for patient outcome, in order to allow a life-saving early colectomy.

The comments generated focused on the extent of CDI in Romania in the last years [2, 3]. The CDI incidence is difficult to be estimated in the absence of a national surveillance system. Fortunately, such a system, coordinated by the National Institute for Public Health was launched in July 2014 [5]. Despite the absence of national incidence data between 2011-2013, we consider that the emergence of CDI in Romania has a real and worrying evolution. Two reasons support this opinion: starting with 2011 in our institution, the largest infectious diseases hospital in Romania, we have documented a sharp increase in cases admitted with CDI [6, 7] and a mortality rate of 6-10% in CDI patients, in contrast with the absence of deaths due to CDI or toxic megacolon before 2011 (unpublished data). The recent emergence of CDI is related to the increased circulation of the more contagious and hypervirulent *C. difficile* ribotype 027. The emergence of CDI in Romania was predictable, seeing the progression of *C. difficile* 027 ribotype in Europe in the last decade, from West to East. The first cases due to 027 at the western border of Romania were communicated from Hungary in 2009 [8]. This geographical progression explains the low incidence of CDI in Romania described by Bauer et al, knowing that they analyzed data collected in 2008 [9].

The 027 ribotype is resistant to fluoroquinolones and it was first signaled in areas with a high consumption of these antibiotics [10]. Moreover, the reduction of quinolones usage was associated with decreased CDI incidence [11]. The high level of fluoroquinolones consumption in Romania (third place in EU in 2012, 3.38 DDD/1000 inhabitants/day) represented a high risk factor for fast dissemination of ribotype 027. The available data indicated a very high prevalence of 027 ribotype in Romania, more than 60% among all *C. difficile* ribotypes [6-7]; in the Bauer data, the highest level was registered for the UK, 33% [9]. The European study EUCLID indicated in 2013 a high proportion of ribotype 027 CDI cases only in Eastern countries (mean, 37.3%), the principal suggested explanation being the low efficacy of measures to contain hospital outbreaks [12].

Finally, we agree that a better diagnosis is a part of the strategy designed to contain the *C. difficile* epidemic in Romania, but the main interventions must be the improvement of antibiotic use, mainly reducing consumption for antibiotics associated with the highest risk for CDI (fluoroquinolones, cephalosporins, carbapenems and clindamycin), and significantly better infection control activities in healthcare institutions.

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Conflicts of interest: None to declare.

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Frameshift mutations of *PRKAG1* gene encoding an AMPK gamma subunit in colorectal cancers

To the Editor,

Recent evidence indicates that AMPK, an important energy-sensing enzyme, has two opposing roles (tumor suppression and promotion) in tumors. Activated AMPK increases cell survival by removing reactive oxygen species [1], whereas activated AMPK is involved in suppressing cellular

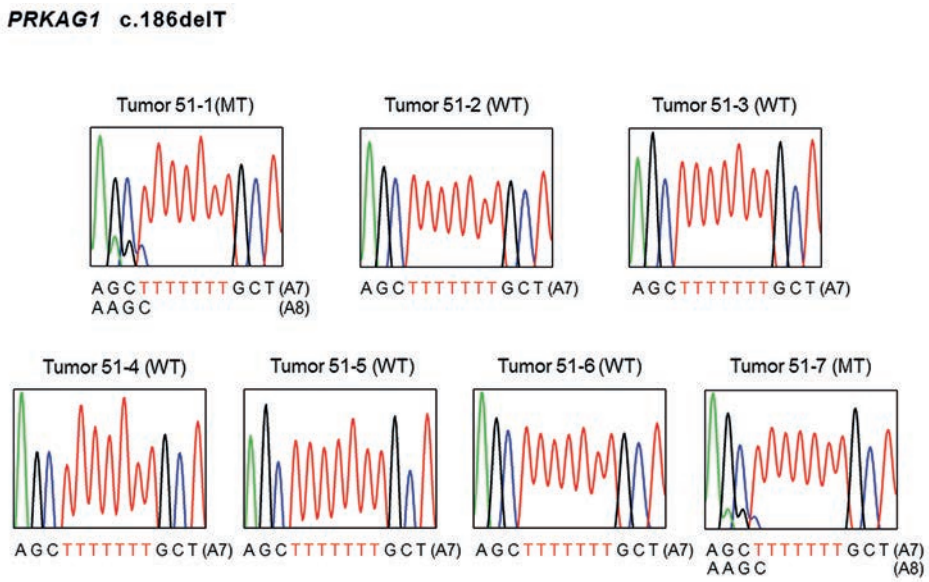

Fig. 1. Intratumoral heterogeneity of *PRKAG1* frameshift mutation in colon cancers. Direct DNA sequencings show *PRKAG1* c.186delT mutation (MT) in a regional biopsy (51-1 and 51-7) and wild-type (WT) *PRKAG1* in the other five regional biopsies (51-2, 51-3, 51-4, 51-5, and 51-6).

proliferation by inhibiting mTOR [2, 3]. *PRKAG1*, encoding one of the three γ proteins of the AMPK, is somatically mutated in 5.5% of colorectal cancers (CRCs) [4]. All of the discovered *PRKAG1* mutations in CRC were missense variants.

In a public genome database (http://genome.cse.ucsc.edu/), we found that human *PRKAG1* had mononucleotide repeats that could be targets for frameshift mutation in CRCs with microsatellite instability (MSI) [5]. To date, however, it is not known whether *PRKAG1* gene is mutationally altered in CRCs with MSI.

In this study, we analyzed a T7 repeat in the *PRKAG1* exon 4 by polymerase chain reaction (PCR)-based single strand conformation polymorphism (SSCP) assay. For this, we used methacarn-fixed tissues of 73 CRCs with high MSI (MSI-H) and 45 CRCs with stable MSI (MSS). We analyzed additional 16 CRCs with MSI-H, from which we had collected four to seven different tumor areas from the same patients. The radioisotope [(32p)dCTP] was incorporated into the PCR products for detection by autoradiogram. The PCR products were subsequently displayed in SSCP gels. After SSCP, direct DNA sequencing reactions were performed in the cancers with mobility shifts in the SSCP [6, 7].

On the SSCP, we observed aberrant bands of *PRKAG1* gene in three CRCs. They were detected in the CRC with MSI-H (3/89), but not in those with MSS. DNA sequencing analyses confirmed that the aberrant bands represented *PRKAG1* somatic mutations, which were a frameshift mutation by duplication of one base [c.186dupT (p.Ala63CysfsX5)] and another one by the deletion of one base [c.186delT (p.Phe62LeufsX4)] in the repeat. Of the three CRCs with the mutations, two were evaluated for the mutational intratumoral heterogeneity (ITH) and one of them showed ITH. This CRC case (#51) showed the *PRKAG1* mutation in two of seven regional biopsies (Fig. 1).

The frameshift mutations detected in the present study would result in premature stops of aminoacid synthesis in *PRKAG1* and hence resemble a typical loss-of-function mutation. The frameshift mutations may alter the AMPK heterotrimeric protein complex and mutated *PRKAG1*-mediated AMPK would be inactivated in CRC with MSI-H, suggesting that AMPK might possibly behave as a tumor suppressor in CRC with MSI-H. We also found ITH of *PRKAG1* mutation in a CRC. Genetic ITH contributes to aggressiveness in cancers and may impede accurate diagnosis and proper selection of cancer therapies [8]. Roles of ITH of *PRKAG1* mutation remain to be clarified in conjunction with the identification of dual biological functions of *PRKAG1* in cancers.

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