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Abstracts

ORIGINAL PAPERS

Noninvasive Detection through REMS-PCR Technique of K-*ras* Mutations in Stool DNA of Patients with Colorectal Cancer

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Abstract

Background and aims. Tumor exfoliated cells that shed into stool are attractive targets for molecular screening and early detection of colon malignancies. Many studies have suggested that the detection of activated *ras* may have diagnostic or prognostic importance. The aim of this study was to establish the suitability for use in diagnostic laboratories of the noninvasive screening test of K-*ras* mutation determination in the stool and its routine prognostic value in colorectal cancer. **Methods.** Paired stool and tissue specimens obtained after polypectomy and colorectal biopsy from 28 patients diagnosed solely by histopathological findings with primary colorectal carcinoma, were prospectively studied for K-*ras* codon 12 mutations by restriction endonuclease-mediated selective (REMS)-PCR. **Results.** DNA was obtained in 28 of tissue samples (100%) and 26 of stool samples (92.8%). K-*ras* codon 12 mutation was seen in 14 (50.0%) paired stool and tissue samples. Mutation detection was possible in 1000-fold excess of wild-type sequence. These results may be important in the design of genetic screening programs, determination of prognosis, early detection and treatment for patients with colon malignancy. **Conclusions.** The sensitivity and specificity of K-*ras* determination on stool-derived DNA in patients with colorectal carcinoma, support the opportunity of a large-scale trial to validate its use as a screening test. REMS- PCR is not labor intensive, but a sensitive, rapid, and robust assay for the detection of point mutations, and was introduced by us in a routine diagnostic laboratory.

Key words

Colorectal cancer – REMS PCR – K-*ras* mutation – stool DNA – noninvasive screening test

Clinical Significance of *p53,* K-*ras* and *DCC* Gene Alterations in the Stage I-II Colorectal Cancers*

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Abstract

Background & **Aims.** Genetic alterations of *p53*, K-*ras* and *DCC* genes have a pivotal role in the colorectal cancer progression. The aim of this study was to clarify the association between K-*ras* mutations, p53 aberrations and *DCC* loss of heterozygosity (LOH), with the patient outcome and tumor characteristics in 43 stage I-II colorectal cancer patients. **Methods.** Mutations in exons 5-8 of the *p53* gene and codon 12 and/or 13 of the K-*ras* gene were assayed by PCR-SSCP and then confirmed by DNA sequencing. *DCC* LOH was studied by PCR-RFLP, while p53 immunohistochemistry was also made. **Results.** Mutations of the *p53* gene were found in 14 (32.5%) tumors. Five (12%) cases showed mutation of the K-*ras* gene. Nuclear staining of p53 was found in 22 (51 %) cases. *DCC* LOH was found in 5 (12%) cases. Cases with guanine to thymine substitution that occurred in K-*ras* codon 12 and *DCC* LOH were found to be more aggressive than other cases with codon 12 mutations or *DCC* wild-type phenotype. Many tumors with p53 over-expression were localized on the left side of the colon (p=0.005). The stage of the tumor was higher in patients who died during the follow-up period, when compared to the ones who have survived. **Conclusions.** Although none of these genetic alterations showed a significant prognostic value, specific mutation of K-*ras* gene and *DCC* LOH phenotype might have a predictive prognostic implication in colorectal cancer. Furthermore, different etiopathogenetic mechanisms might be involved in the tumorigenesis of the left and right colon.

Key words

Colorectal cancer - p53 - K-*ras* - *DCC* LOH - prognosis

Long Term Follow-Up of a Large Cohort of Inactive HBsAg (+)/ HBeAg (-)/ anti-HBe (+) Carriers in Greece

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Abstract

Aim. To investigate the long-term outcome and the risk of progression to chronic hepatitis B in inactive hepatitis B surface antigen carriers. **Material and methods.** A total of 307 HBsAg (+)/HBeAg (-)/antiHBe (+) subjects with initially normal alanine aminotransferase (ALT) levels and undetectable/ low serum HBVDNA with hybridization assay and later with PCR ($\leq 10^5$ copies/ml), were followed-up every 6 months for a period of 3 to 21 years (7.45$\pm$3.75 years). **Results.** 234 out of the 307 HBsAg (+) patients (76.2%) had persistently normal ALT and undetectable / low ($\leq 10^5$ copies/ml) HBVDNA during follow-up. In 73 patients (23.8%), a reactivation of the disease with elevated ALT and positive HBVDNA ($>10^5$ copies/ml) was recorded during the follow up. Thirty-five out of 73 patients underwent liver biopsy, while 22 of them received treatment. Twenty-four patients (7.8%) lost HBsAg after a mean of 7.4$\pm$3.6 years. Regarding the complications of chronic hepatitis B, only one patient developed compensated cirrhosis and no one developed HCC. **Conclusions.** Our results show that in almost 24% of inactive chronic hepatitis B carriers reactivation of the disease may occur even after many years. However the risk of liver-related complications is very low in these subjects.

Key words
Chronic hepatitis B – HBVDNA - inactive carrier

Efficacy, Tolerability and Predictive Factors for Early and Sustained Virologic Response in Patients Treated with Weight-Based Dosing Regimen of PegIFN α-2b and Ribavirin in Real-Life Healthcare Setting

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Abstract

Background and aim. Increasing evidence to date highlights that individualized treatment regimens with pegylated interferon (PegIFN) and ribavirin represent a better approach for patients nowadays showing negative predictive factors for sustained virological response. The aims of this study were to assess the rate of early (EVR) and sustained virological response (SVR), tolerability and baseline predictive factors associated with EVR and SVR in patients with chronic hepatitis C treated with individualized weight-based dosing regimen for both PegIFN -2b and ribavirin. **Methods.** The observational analysis included 234 consecutive patients with chronic hepatitis C genotype 1 treated with PegIFN -2b and ribavirin on an out-patient basis between January 2003 – March 2006. **Results.** The mean age of the study group was 49.5 years, and 35% were male patients; the group was slightly overweight (mean BMI=26.5 kg/m^2). EVR was achieved in 84.6% (198/234 patients). The end-of-treatment and sustained biochemical responses were 76.3% and 66.1%, respectively. At the end of follow-up, an overall intent-to-treat SVR was achieved by 71 of 127 patients (in 55.9%). Lower baseline (<1,000 000 IU/mL) HCV viral load was the only predictive factor associated with EVR (p=0.04); absent or mild fibrosis (F0-1) and a low histological activity (HAI<8) were independently associated with SVR. Side effects resulted in PegIFN and ribavirin dose reductions in 9.4% and, respectively, 18.1%, but definitive discontinuation of therapy was necessary only in 8.7% of patients. **Conclusion.** PegIFN -2b and ribavirin can be safe and successful when using a weight-based dosing regimen, leading to high response rates even in overweight patients.

Key words
Chronic hepatitis C - weight-based dosing regimen - PegIFN -2b - early virologic response - sustained virologic response

The FibroTest Value in Discriminating between Insignificant and Significant Fibrosis in Chronic Hepatitic C Patients. The Romanian Experience

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Abstract

Aim. To assess the diagnostic value of FibroTest to discriminate between insignificant and significant fibrosis in order to avoid the liver biopsy currently used for selection of chronic hepatitis C patients eligible for antiviral therapy. **Patients and methods.** A retrospective study was carried out in 206 chronic hepatitis C patients with liver biopsy performed before starting antiviral therapy and concomitant serum stored at -80°C. Liver fibrosis was evaluated according to the METAVIR scoring system on a scale of F0 to F4. Biochemical markers assessed were: alpha 2 macroglobulin (2-MG), apolipoprotein A1 (Apo-A1), haptoglobin (Hapto), gamma-glutamyltransferase (GGT), total bilirubin (TB). The FibroTest score was computed after adjusting for age and gender. Predictive values and ROC curves were used to assess the accuracy of FibroTest results. **Results.** 2-MG, apo-A1, Hapto and gender were independent predictors for significant fibrosis. For FibroTest the observed area under ROC (ObAUROC) for the discrimination between minimal or no fibrosis (F0-F1) and significant fibrosis (F2-F4) was 0.782 (± 95CI: 0.716-0.847) for a cutoff value 0.47. The sensitivity (Se), specificity (Sp), positive predictive value (PPV) and negative predictive value (NPV) of the FibroTest to differentiate significant from insignificant fibrosis were 80.2; 63.2; 78.9 and 65.8, respectively. The adjusted AUROC (AdAUROC) according to the prevalence of each individual stage of fibrosis was 0.856. **Conclusion.** FibroTest could be an alternative to biopsy in most patients with chronic hepatitis C. It requires a strict adherence and observance of the technical recommendations for the assays of biochemical markers in order to avoid analytical variability.

Key words

Liver fibrosis - serum biochemical markers - FibroTest

Therapeutic Options in Non-Alcoholic Steatohepatitis (NASH). Are all Agents Alike? Results of a Preliminary Study

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Abstract

Aim. The evaluation of the efficacy of ursodeoxycholic acid (UDCA), pentoxifylline, losartan, and atorvastatin in non-alcoholic steatohepatitis (NASH) treatment. **Method.** 48 patients (25 males/23 females, aged 55 $\pm$ 7.54 years) with histologically confirmed NASH were enrolled between 2001 and 2005. The batch was divided into four groups: A (10 dyslipidemic patients, receiving atorvastatin 10 mg/day), P (13 nonhypertensive/ nondyslipidemic patients receiving pentoxifylline 400 mg bid), L (12 hypertensive patients, treated with losartan, 50 mg/day) and U (13 nonhypertensive patients receiving UDCA 15 mg/kg/day). Mean duration of treatment was 37.8 $\pm$ 5.4 weeks. Body mass index, liver biopsy and serum level of alanin-aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phos-phatase (AP), total cholesterol (TC) and triglycerides (TG) were determined at inclusion and at the end of treatment. Liver biopsy samples were evaluated for necroinflammation, steatosis and fibrosis (Brunt's score). **Results.** In group A, a significant reduction of ALT, GGT, TC and AP was noticed. Histology showed diminished steatosis, but no improvement of fibrosis and necroinflammation. In

groups P and L we found a reduction of mean ALT and GGT levels and necroinflammatory score. Group U presented a significant reduction in ALT and GGT levels, without improvement in steatosis, necroinflammation or fibrosis. **Conclusion.** Atorvastatin and losartan proved to be efficient in the treatment of dyslipidemia- and hypertension-associated NASH, by improving both biochemical parameters and steatosis/ necroinflammation. Pentoxifylline showed similar efficacy in non-hypertensive/non-dyslipidemic patients, while UDCA did not improve the histological score although it improved the biochemical parameters.

Key words

Non-alcoholic steatohepatitis - pentoxifylline - losartan- atorvastatin - ursodeoxycholic acid - NAFLD activity score

Glycogen Storage Disease Type I - between Chronic Ambulatory Follow-up and Pediatric Emergency

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Abstract

Background and aims. To describe the characteristics of patients with type I glycogenosis, the presentation types, the main clinical signs, the diagnostic criterias and also the disease outcomes on long term follow-up. **Methods.** The study group consisted of 6 patients (medium age 3 years 6 months) admitted in hospital between 2001 and 2005 and followed-up for 1 to 5 years. The sex ratio was 1:1. **Results**. The referral reasons varied from hepatomegaly incidentally discovered (3 of 6 patients) to abdominal pain (4 of 6 patients), growth failure (3 of 6 patients), symptoms of hypoglycemia (3 of 6 patients), recurrent epistaxis (1 patient). Hepatomegaly was present in all cases. Biological profile: hypoglycemia, increased transaminase values, hypertri-glyceridemia, lactic acidosis, normal uric acid levels. Two patients had neutropenia and other two had increased glomerular filtration rate. Liver biopsy showed glycogen-laden hepatocytes and markedly increased fat. Four patients had type Ia and 2 patients type Ib glycogenosis. The therapy consisted of: diet, ursodeoxycholic acid, granulocyte colony-stimulating factor, broad spectrum antibiotics for those with type Ib glycogenosis. The follow-up parameters were clinical, biological, imaging. Metabolic interventions and antiinfectious therapy were necessary. All patients are alive, two of them on the waiting list for liver transplantation. **Conclusions**. Glycogen storage disease type I is a rare condition, but with possible life-threatening consequences. It has to be kept in mind whenever important hepatomegaly and/or hypoglycemia are present.

Key words

Glycogen storage disease type I - hypoglycemia - children - hepatomegaly

Pre-emption Dimensional Study for Obtaining Statistically Significant Results for the Variation of Glutamyl-Transferase During Ovarian Stimulation

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Abstract

Background. Ovarian stimulation with gonadotropins/ gonadotropin releasing factor agonists (a-GnRH), largely used currently for infertility treatment, can induce hepatic effects, demonstrated only in animals or in women with hyperstimulation syndrome. **Aim.** We wanted to estimate the number of included patients for which the variation of gglutamyl-transferase (GGT) during and due to ovarian stimulation could be sustained by statistic validation. **Method.** In 23 consecutive patients, aged 23–45 years (mean 32.6 ± 7.4 years) included in a fertility program, busereline, an a-GnRH was started the first day of the cycle and followed from the 14th day with human menopausal gonadotropin. Ovulation was triggered with human chorionic gonadotropin. GGT was measured in the serum the first day (control), in the 14th, 19th, 24th day, the day before the triggering of the ovulation and two days after that. The statistic study used a distribution analysis (Student *t* test) – BMDP, SAS 6.0 and EpiInfo 5 software and calculated the necessary number of measurements in order to obtain significant (95%) variation for GGT (actual and = 5%). **Results and con-clusions.** The proper number of determinations which statistically support a possible significant difference is 29-38 and 46 for a 5% difference. The considered suppositions are able to support a correct estimation.

Key words

Dimensional study - gglutamyl-transferase - ovarian stimulation - gonadotropins - gonadotropin releasing factor agonists

REVIEWS

Update on the Management of Refractory Coeliac Disease

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Abstract

True Refractory Coeliac Disease (RCD) is being currently defined as persisting or recurring villous atrophy with crypt hyperplasia and increased intraepithelial lymphocytes in spite of a strict gluten free diet for more than 12 months or when severe persisting symptoms necessitate intervention independent of the duration of the dietary therapy. Currently two categories of RCD are being recognized: type I without aberrant T-cells and type II with aberrant T-cells detected by immunophenotyping by flowcytometric analysis or immunohistology of the intestinal mucosa. Establishing the diagnosis of RCD requires exclusion of other causes of villous atrophy and malignancies that may complicate coeliac disease. In contrast to patients with a high percentage of aberrant T cells, patients with RCD type I seem to profit from an immunosuppressive treatment. In cases of RCD II with persistent clinical symptoms and/or high percentage of aberrant T cells in intestinal biopsies in spite of a corticosteroid treatment, more

aggressive therapeutic schemes should be considered. However, no therapy seems to be curative in RCD II. Cladribine (2-CDA) seems to have some role in the management of these patients. More recently, high dose chemotherapy followed by autologous stem cell transplantation has been used in patients resulting in a dramatic improvement in the clinical, laboratory, histopathological and immunological parameters. This review provides an overview of the currently available diagnostic and therapeutic methods in a complicated form of coeliac disease.

Key words

Coeliac disease – refractory coeliac disease – pathogenesis – diagnosis - therapy

Extrahepatic Manifestations of Chronic HCV Infection

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Abstract

Several extrahepatic manifestations have been reported in the natural history of hepatitis C virus infection (HCV). Up to 40-74% of patients infected with HCV might develop at least one extrahepatic manifestation during the course of their disease. Mixed Cryoglobulinemia (MC) is the most known and studied syndrome associated with HCV infection. It is a systemic vasculitis that may involve the skin, kidney and nervous system. A frequent reported association is that between HCV infection and non-Hodgkin lymphoma. The cryoglobulinemia may be the intermediary disorder, in fact some persistent forms of cyoglobulinemia can switch over to a more aggressive haematologic disorder. As compared to cutaneous vasculitis described in MC, HCV infection has been associated with dermatological disorders such as porphyria cutanea tarda and lichen planus. Thyroid disease (usually hypothyroidism) is commonly seen in people with HCV. Up to 25% have thyroid antibodies. Several studies described a correlation between HCV and lympho-cytic sialoadenitis, similar to sialoadenitis associated with idiopathic Sjögren syndrome, but we can define as “pseudo- Sjögren syndrome” the one associated with HCV infection, because it shows several differences in the idiopathic form. In the course of chronic HCV infection, a common obser-vation are rheumatological symptoms such as polyarthritis. The clinical pattern of joint involvement in the course of HCV infection varies from a rheumatoid arthritis-like form (very rare), to a non erosive oligoarthritis involving the large-sized and middle joints.

Key words

Extrahepatic manifestations - chronic HCV infection - mixed cryoglobulinemia - B-cell - non-Hodgkin lymphoma - pseudo-Sjögren syndrome

CLINICAL IMAGING

The Applicability of Radial Endoscopic Ultrasonography in Pancreatic Diseases

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Abstract

Radial endoscopic ultrasonography (EUS) is used in pancreatic pathology for diagnosing such diseases as: chronic pancreatitis, cancer of the pancreas, neuroendocrine tumors, and the non-inflammatory cyst lesions of the pancreas. For diagnosing chronic

pancreatitis, the use of more than four criteria of endoscopic diagnosis offers a high diagnostic accuracy. In the case of tumors of the pancreas, EUS detects small-size tumors, having a major diagnostic value in the preoperative staging and in assessing the tumor resectability. EUS can best identify insulinoma, while for the differential diagnosis of cystic neoplastic lesions data provided by radial EUS are not sufficient. In this respect, intracyst fluid analysis is required to identify malignant lesions or those having malignancy potential.

Key words

Pancreas - endoscopic ultrasonography - EUS - cystic lesions - neuroendocrine tumors - chronic pancreatitis

Evaluating the Liver Tumors Using Three-Dimensional Ultrasonography. A Pictorial Essay

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Abstract

The liver tumors represent a major public health issue. Among the imaging techniques, ultrasonography remains an important diagnostic method, taking into consideration its large availability, the non-invasive and repetitive characteristics and the low cost. Its current limits can be surpassed, at least at some extent, by using some new techniques such as the 3D ultrasonography. 3D ultra-sonography provides several advantages: increases the efficiency of the liver tumor screening by using the multislice function, allows a better time and human resource management, the precise location on three axes of the tumor and the exact assessment of the distances relative to the main vessel structures of the liver. It is also able to characterize the tumor texture and to sum up the total volume of liver masses, with applications in post-chemotherapy follow-up.

Key-words

Liver tumors – imaging techniques – three-dimensional ultrasonography

SURGICAL TECHNIQUE / CASE REPORT

Celiac Artery Compression Syndrome: Successful Utilization of Robotic-Assisted Laparoscopic Approach

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Abstract

Median arcuate ligament (MAL) syndrome, also known as the celiac axis compression syndrome (CACS) is rare, and a topic of ongoing academic controversy. CACS is a diagnosis of exclusion, characterized by the clinical triad of postprandial abdominal pain, weight loss, and vomiting. The classic management of CACS involves the surgical division of the MAL fibers. We report successful treatment of a 23-year-old woman with CACS utilizing the da Vinci Surgical System (Intuitive Surgical, Sunnyvale, California) via robotic-assisted minimally invasive surgical division of the MAL. To our knowledge

this is the first report of this modality used in the treatment of the celiac axis compression syndrome.

Key words

Celiac axis compresion syndrome – robotic-assisted – minimally invasive surgery

CASE REPORTS

A Case of Portal Cavernoma - Associated Essential Thrombocythemia

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Abstract

Portal cavernoma is a rare complication of portal vein thrombosis and may be a component of the myeloproli-ferative hematologic disorders. In the present case report, although the symptoms induced by portal hypertension and portal biliopathy had a concordant evolution with the portal cavernoma, the peripheral thrombocythosis and the medullar expression of essential thrombocythemia became relevant at 18 months.

Key words

Portal vein thrombosis – portal cavernoma – essential thrombocythemia

Problems in Diagnosing Lymphoma of the Pancreas with Computed Tomography. A Case Report

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Abstract

Primary lymphoma of the pancreas is a rare form of extranodal lymphoma accounting for less than 0.5% of pancreatic tumors. Percutaneous fine-needle aspiration of the pancreas with histopathological examination and immunohistochemical assay confirm the diagnosis. A 73 year old male presented with recurrent pancreatic type abdominal pain with significant weight loss over 1 year. He was pale with ill defined epigastric mass. Contrast enhanced CT showed an ill defined poorly marginated non enhancing hypodense mass lesion involving the body of the pancreas. CA 19-9 was normal. CT guided aspiration cytology was suggestive of hemolymphoid malignancy. Immunohisto-chemistry was positive for Leukocyte Common Antigen and CD 34. It was negative for CD3 and CD 20 indicating an undifferentiated lymphoma. Patient received two sessions of chemotherapy and was followed-up.

Key words

Pancreatic lymphoma - contrast enhanced CT - fine needle aspiration cytology - immunohistochemistry

Natural Evolution of an Intraductal Papillary Mucinous Neoplasm of the Pancreas. A Case Report

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Abstract

Intraductal papillary mucinous neoplasms include a large spectrum of lesions communicating with the Wirsung duct, having a variable invasiveness from benign or borderline, to malignant (carcinoma in situ and invasive cancer). Final diagnosis is based on endoscopic ultrasound (EUS)-guided fine needle aspiration and histopathologic exam of surgical specimens. We present the case of a 28-year-old woman, with several episodes of acute recurrent pancreatitis in the past 6 months, admitted for dyspepsia, nausea and loss of appetite. Imaging studies (transabdominal ultrasonography, CT scanning, MR cholangiopancreatography) showed a macrocystic, multilocular, corporeal tumor, communicating with the retrograde dilated Wirsung duct. EUS revealed hypoechoic material inside the cysts, raising the suspicion of an intraductal papillary mucinous neoplasm. Diagnosis was confirmed by EUS-guided fine needle aspiration, which found columnar mucinous cells within a mucin-rich fluid. The imaging evaluation was repeated after two years, showing a rapid evolution of the tumor. The patient refused surgical exploration and caudal pancreatectomy. In the context of the absence of clinical symptoms, the indolent evolution of these tumors and the excellent prognosis after resection, we consider that early identification and regular follow-up by EUS with fine needle aspiration is imperative, especially because of the limited success of other imaging methods.

Key words

Intraductal papillary mucinous neoplasm – MRCP - endoscopic ultrasound - EUS-FNA

Unusual Presentation and Complication of Caustic Ingestion. Case Report

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Abstract

Caustic substances cause tissue destruction through liquefaction or coagulation reactions and the intensity of destruction depends on the type, concentration, time of contact and amount of the substance ingested. We report an unusual presentation and complication of caustic ingestion in a patient, who accidentally ingested sodium hydroxide. Our patient presented respiratory failure soon after admission and developed necrotizing esophagitis with progression to esophageal stenosis, which required surgical treatment. The complications were related to the amount of caustic soda ingested.

Key words

Caustic soda - necrotizing esophagitis - respiratory failure - caustic stenosis

A Pyogenous Gastric Abscess that Developed Following Ingestion of a Piece of a Wooden Skewer: Successful Treatment with Endoscopic Incision

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Abstract

A 62-year-old man with a medical history of duodenal ulcer was referred to our department for endoscopy, because of epigastralgia associated with mild anorexia. At endoscopy, a large protruding lesion with the appearance of a submucosal tumor was present at the posterior wall of the gastric body. Trying to perform an aspiration needle biopsy for cytological study, we observed the outflow of pus from the puncture point. An incision, 1 cm in length, was performed via a needle-knife sphincterotome, which led to drainage of the abscess and disappearance of symptoms. A 2.5 cm long piece of a wooden skewer was found to be embedded into the area of the bulge and was retrieved.

Key words

Gastric abscess - wooden skewer - endoscopic treatment