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Case Report

Epstein–Barr virus-associated gastric carcinoma 33 years after kidney transplantation

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Abstract

Epstein–Barr virus-associated gastric carcinoma (EBVaGC) is a unique type of gastric cancer, defined as the presence of EBV in gastric tumour cells, usually identified by *in situ* hybridization. A poorly differentiated gastric adenocarcinoma was detected in a kidney recipient 33 years after transplantation. Neoplastic epithelial cells were EBV positive by *in situ* hybridization. Gene sequencing confirmed the amplicon specificity, and real-time polymerase chain reaction quantified 2 600 000 genomes/μL DNA in neoplastic tissue. No cases of EBVaGC have been reported in solid organ transplants, thus this is the first case of *de novo* EBVaGC arising in a 65-year-old renal transplant recipient.

Keywords: Epstein–Barr virus; gastric cancer; kidney transplantation; molecular analyses

Background

Epstein–Barr virus (EBV) is associated with several types of malignancies, such as lymphoma, nasopharyngeal carcinoma and gastric carcinoma [1]. EBV-associated gastric carcinoma (EBVaGC) is a unique type of gastric cancer consisting of neoplastic cells with monoclonal EBV, first described in 1990 [2]. No cases of EBVaGC have been reported in solid organ recipients, and only one case has been described in a bone marrow transplant patient [3]. We report a case of EBVaGC arising in a renal transplant recipient 33 years after transplantation.

Case report

A 65-year-old man was admitted to a peripheral hospital in November 2008 complaining of fatigue and dyspnoea on exertion. His medical history reported renal transplantation at the age of 32 for renal failure following paint fixative exposure. Splenectomy was performed during transplantation and his immunosuppressive regimen included corticosteroids and azathioprine. Three days after transplantation, the patient experienced an episode of acute cellular rejection, which was treated with corticosteroid pulses, graft irradiation and antilymphocytic serum. Afterwards, the graft always showed an excellent function (creatinine clearance >60 mL/min, proteinuria absent, no further rejection episodes in the follow-up). On admission, as the faecal occult blood test was positive, a gastroscopic examination was performed which revealed diffuse redness of the gastric mucosa. Multiple endoscopic biopsies were taken and a diagnosis of poorly differentiated adenocarcinoma from the gastric body was established. The patient was transferred to our centre for staging and surgery. Computerized tomography scan showed diffuse thickening of the posterior wall of the gastric body and fundus, without metastases in other organs. Total gastrectomy and regional lymphadenectomy were performed. EBV DNAemia was <300 genomes/100 000 lymphocytes. Unfortunately, no information was available about any EBV/CMV mismatch as the patient had been transplanted outside Italy many years before. The post-operative course was uneventful and the patient was discharged 20 days after surgery. The immunosuppressive regimen was minimal at the time of diagnosis (azathioprine 50 mg/day and prednisone 8 mg/day) and was therefore left unchanged. In the follow-up, an upper digestive barium test showed regular esophageal-jejunal anastomosis. The patient had regular food intake and his S-creatinine remained normal (60 μmol/L). One year after surgery, upper GI haemorrhage occurred, and in December 2009 the patient died of recurrent disease.

Pathological findings

At gross inspection, the stomach showed diffuse thickening and rigidity of the wall, enlarged rugal folds and a 4 × 3 cm intraluminal polypoid mass (Figure 1A). Histology revealed a poorly differentiated diffuse adenocarcinoma involving the whole wall, associated with extensive fibrosis and surface erosion. Mononuclear inflammatory cell infiltration was present. No lymph nodes or resection margins were involved. A final diagnosis of EBVaGC stage IIA was

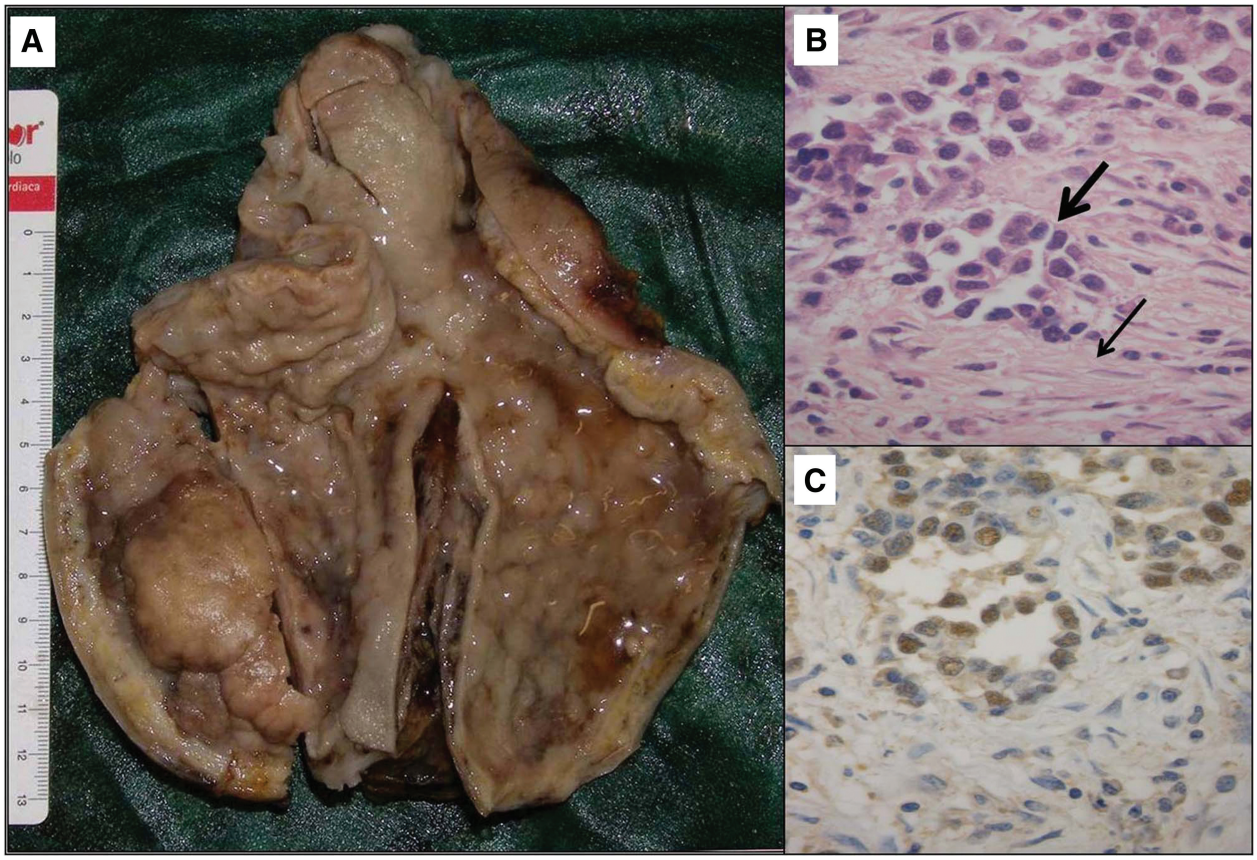

Fig. 1. Macroscopic view of the stomach showing diffuse thickening and rigidity of the wall, enlarged rugal folds and a 4 × 3 cm intraluminal polypoid mass (**A**). Histological view showing small irregular nests of malignant cells often with acinar pattern (arrow). Note the extensive peritumoural desmoplastic reaction (thin arrow). Haematoxylin and eosin, ×100 (**B**). EBV genomes are well detected within the nuclei of almost all neoplastic epithelial cells. *In situ* hybridization for Epstein–Barr early RNA EBER, ×100 (**C**).

made. Immunohistochemistry characterized tumour cells as CAM 5.2 positive, CK7 and CK20 negative. Inflammatory infiltrates consisted of mixed lymphomonocytes including CD3+ T lymphocytes, CD20+ B lymphocytes, CD68+ monocytes and CD79a+ plasma cells. *In situ* hybridization (ISH) for Epstein–Barr early RNA EBER (Epstein–Barr encoded RNAs) showed that EBV genomes were present within the nuclei of almost all neoplastic epithelial cells but not in the inflammatory mononuclear cells (Figure 1B,C). EBV presence was confirmed by polymerase chain reaction (PCR) using specific primers for EBNA1 (Epstein–Barr nuclear antigen 1) and the direct cycle sequencing of EBV PCR product (100% identity with EBV genome strain B95-8, a type 1 strain). Quantitative real-time PCR carried out using the artus® EBV TM PCR KIT showed a very high number of copies in neoplastic tissue (2 600 000 copies/µL DNA) (Figure 2A,B).

Discussion

De novo cancer is a major complication of renal transplant that causes significant short- and long-term mortality. It is responsible for 20% of deaths in renal transplant patients every year and even for 30% of deaths in those patients who have a follow-up time of more than 20 years [4]. Despite the high propensity to develop tumours, gastric cancer is rare in grafted patients: only 33 gastric cancers have been described among the 7000 malignancies reported to the Israel Penn International Transplant Tumour Registry [5] and 7 cases of 172 kidney transplants were reported in the North Italian Transplant registry [6]. Azathioprine is one of the immunosuppressive agents most often linked with post-transplant malignancies [7] as it directly promotes cancer through several mechanisms (e.g. leading to the mutagenic accumulation of 6-thioguanine in patient DNA or selecting clones with DNA mismatch repair deficiencies). A recent long-term randomized follow-up study suggests that azathioprine- and cyclosporine-based regimens are associated with similar overall long-term cancer risks. Thus the total burden of immunosuppression, more than the single agent, appears to be responsible for the increased oncogenic risk [8]. Immunosuppressive therapy is also known to be related to a higher susceptibility to viral infections, which could have oncogenic properties.

EBVaGC, whose prevalence is 1.3–20% in gastric carcinomas occurring in the general population [9], is defined as the presence of EBV in gastric tumour cells and is always identified by performing EBER ISH. To the best of our knowledge, no cases of EBVaGC have been reported

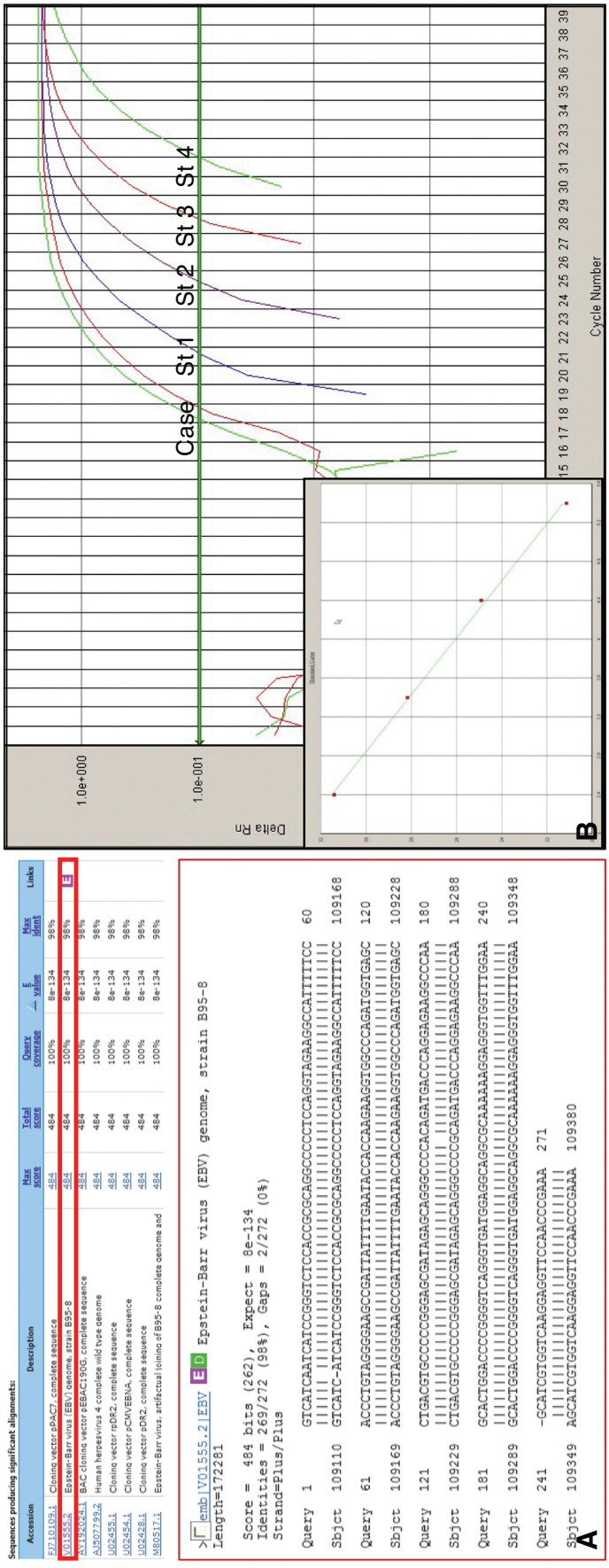

Fig. 2. Direct cycle sequencing of EBNA1 showed that the EBV genome was 100% of the B95-8 strain, a type 1 strain (V01555.2) (**A**). Quantitative real-time PCR showed a very high number of EBV genome copies (2 600 000 copies/μL DNA) (**B**).

in solid organ transplanted patients. Therefore, this is the first case of *de novo* EBVaGC arising in a 65-year-old renal transplant recipient. A previous case has been reported, but it was a case of early onset after non-myeloablative haematopoietic stem cell transplantation for myeloma with subsequent strong immunosuppression due to a severe graft-versus-host disease [3]. Here, we have demonstrated EBV in neoplastic tissue by positive ISH for EBER, gene sequencing for EBNA1 and real-time PCR, revealing a very high number of EBV genomes in spite of a low DNAemia. EBV PCR in the peripheral blood is a useful screening tool in transplant recipients; however, sometimes EBV blood level does not represent a mirror of EBV tissue presence, as was in our case. Thus, EBVaGC could be underestimated since gastric cancers are not routinely investigated using molecular viral analyses on tissue.

EBV has frequently been associated with the hypermethylation in promoters of various tumour-related genes which can lead to uncontrolled cell proliferation and viral propagation.

Whether genetic alterations precede EBV infection due to long-time duration of immunosuppressive therapy or if these are consequences of EBV infection itself remains to be established through further studies. In particular, it could be interesting to develop a careful molecular tissue investigation of gastric carcinomas which develop in transplanted patients in order to obtain a precise incidence in this patient population. It could be useful for nephrologists or other specialists involved in the monitoring and care of renal transplanted patients to suggest a specific diagnostic screening in high-risk patients. In particular, patients with organ-specific symptoms (nausea, anaemia, weakness, gastrointestinal bleeding, etc.) should be promptly subjected to gastrointestinal endoscopy together with molecular EBV/CMV screening of tissue and annual blood screening.

Conflict of interest statement. None declared.

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