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Endoscopic ultrasound-guided fine needle tissue acquisition biopsy samples do not allow a reliable proliferation assessment of gastrointestinal stromal tumours

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ABSTRACT

Background: Current prognostication of gastrointestinal stromal tumours is validated on/applies to resected tumours, mainly because surgery is recommended whenever possible. However, pre-treatment prognostication is increasingly warranted, considering the follow-up strategy recently admitted for expectedly low-risk tumours and the possible distinctive molecular features/spontaneous regression of some small cases.
Aims: To investigate whether endoscopic ultrasound-guided fine-needle tissue acquisition-biopsies reflect prognosticators of resected gastrointestinal stromal tumours, for possibly developing reliable pre-treatment prognostic criteria.
Methods: The applicability/reliability of mitotic index/5 mm^2 and MIB1 proliferative index/1000 cells were tested in 35 endoscopic ultrasound-guided fine-needle tissue acquisition-biopsies diagnosed as gastrointestinal stromal tumour, subsequently resected without intervening therapy, consecutively collected in thirty months. Size and mitotic/proliferative indexes were compared with resection specimens. The feasibility of bioptic genotyping was also tested.
Results: 35 patients were studied (45.7% males; mean age 61.6 years, range 26–83 years). Mitotic/proliferative indexes were determinable in 68.6%/88.6% of biopsies, respectively; they were nevertheless underestimated, as happened with endoscopic ultrasound-determined tumour size. Bioptic genotyping revealed reliable.
Conclusions: Endoscopic ultrasound-guided fine-needle tissue acquisition does not reliably reflect gastrointestinal stromal tumours' proliferation and size. Alternative parameters should be validated for a pre-surgical prognostic classification. Considering the emerging potentially prognostic genetic markers in gastrointestinal stromal tumours, the reliability of bioptic genotyping is a promising result.

1. Introduction

Gastrointestinal stromal tumours (GISTs) are the most common mesenchymal tumours and subepithelial lesions in the gastrointestinal tract [1–4]. Current prognostication of GISTs applies to resected tumours and is based on tumour site, size and mitotic index (MI) as determined on 5 mm^2 [5,6]. A reliable preoperative prognostication system of GISTs has not been developed so far, mainly because the uncertain clinical behaviour of these tumours as a group rendered surgical excision their recommended standard treatment. However, the need to predict the biology of unresected GISTs does exist in some cases, and the fraction of GISTs eligible to strategies alternative to surgery is increasing [5,7,8], thus justifying proposals of pre-treatment classifications for improving the management of GIST patients [9].

We recently proved that the technique named endoscopic ultrasound (EUS)-fine needle tissue acquisition (FNTA) (EUS-FNTA)

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using a 19-gauge needle without the stylet performed in conjunction with the forward-viewing EUS scope is very effective to reach a definitive histologic diagnosis in a large series of Subepithelial lesions [10]. The forward-viewing EUS scope was choosen for this indication because its design with the frontal exit of the needle allows the tip of the scope to lean directly against the subepithelial lesion to be sampled and then to maximize transfer of the force to the target site, with penetration of the lesion accomplished with a sharp push of the needle, allowing its advancement inside the lesion [10].

The primary aim of the present study was to evaluate the feasibility of proliferative indicators (MI/5 mm^2 and MIB1 proliferative index per 1000 cells –PI–) in EUS-FNTA samples of GISTs and their reliability as compared with surgical specimens. Secondary aims were to assess: (1) whether data from EUS-FNTA can reliably reflect surgical-based risk classes; (2) the feasibility and reliability of genetic analysis of *KIT* and *PDGFRA* genes, fundamental for GIST pathogenesis, prognostication and therapy profiling [1,6,11], in EUS-guided FNTA-biopsies.

2. Materials and methods

2.1. Study design

This study represents a retrospective analysis of a prospectively collected database. The pathology and EUS databases of Policlinico A. Gemelli Hospital of the Catholic University in Rome were reviewed to identify all consecutive patients who, between January 2009 and June 2013, were surgically resected, without previous tyrosin-kinase inhibitor treatment, following a diagnosis of GIST after EUS-FNTA for subepithelial lesions of the gastrointestinal tract. In the EUS-FNTA specimens retrieved, the physical features (i.e. number and size of fragments, total length and section area) were determined, as well as MI/5 mm^2 and PI whenever possible. Tumour sizes (determined by EUS in case of FNTA biopsies –EUS-size–), MI/5 mm^2 and PI were compared between FNTA-biopsy and resection specimen in corresponding tumours.

2.2. EUS examination

All EUS procedures were performed under conscious or deep sedation using the forward-viewing EUS scope (XGIF-UCT160J-AL5; Olympus Medical Systems Europe, Hamburg, Germany), as described previously [10]. EUS-FNTA was done using a standard 19-gauge needle (Echotip Ultra; Cook Medical Inc., Bloomington, IN, USA). The needle was prepared before insertion into the working channel of the echoendoscope by removing the stylet and attaching a 10-mL syringe to its proximal end, preloaded with 10 mL of negative pressure. The needle was then advanced a few millimetres into the target lesion under EUS guidance. After opening the lock of the syringe to apply negative pressure, three back-and-forth motions were made with the needle inside the target lesion, together accounting for one needle pass. The lock of the syringe was then closed and the needle removed. The collected specimens were immediately placed in 10% formalin. There was no pathologist on site during the endoscopic procedure. No early or late complications related to the EUS-FNTA procedures were observed.

2.3. Histology and immunohistochemistry

Sections from formalin-fixed, paraffin-embedded specimens were stained with haematoxylin/eosin. The antibodies used for immunohistochemistry were: CD117 (rabbit polyclonal) and MIB1 (M7240) (reacting with Ki-67 antigen) (DAKO, Glostrup, Denmark, 1:400 and 1:100, respectively), and DOG1 (Spring Bioscience, Pleasanton, CA, USA, rabbit polyclonal, 1:100). Deparaffination was performed with Dako Target Retrieval Solutions High pH (K6002) for MIB1, and low pH (K8005) for CD117 and DOG1, for 15 min at 65 °C, using the Dako pretreatment (PT) link. For MIB1 and DOG1, antigen retrieval was then performed in the same solutions for 20 min at 98 °C. After blocking of endogenous peroxidase activity with Dako EnVision Flex+ (K8002) for 5 min, tissue sections were incubated with primary antibody diluted in Dako antibody diluent (K8006) for 40 min at room temperature. The reaction was visualized using Dako EnvisionFlex+ mouselink for 15 min followed by diaminobenzidine for 10 min. The sections were counterstained with haematoxylin for 1 min.

2.4. Determination of mitotic and proliferative indexes

MI/5 mm^2 and PI immunohistochemistry were determined in FNTA-biopsies and resection specimens as follows: MI/5 mm^2 was assessed counting the number of mitoses in 21 high-power fields (HPFs) at a magnification of ×400 using an Olympus microscope with a ×40 plan objective (0.24 mm^2), corresponding to 5 mm^2. PI was assessed counting the percent MIB1 nuclear staining on at least 1000 tumoral cells; in detail, cells with and without stained nuclei were counted in delimited areas of microphotographs taken from the investigated tumours; the count went on until the threshold of 1000 cells was trespassed by adding the cells of the last counted area. For both MI/5 mm^2 and PI, when areas with different proliferation activities were identified, the count was performed in the most active area; the counted fields were consecutive as much as possible (i.e., counts had to be shared among different fragments in fragmented FNTA-biopsy samples since no single fragment allowed to reach 21 HPFs or >1000 cells).

2.5. Definition of EUS-FNTA-based preoperative "virtual" risk class

A preoperative "virtual" risk class was evaluated by combining the anatomical site, the EUS-size and FNTA-biopsy-assessed MI/5 mm^2. The resulting risk class for each GIST was then compared with the actual, postsurgical risk-class determined on the corresponding resected specimen [5,6].

2.6. Genetic analyses

Three 10-μm slides were cut from paraffin-embedded tissues, treated twice with xylene, and then washed with ethanol. DNA was extracted using the QIAamp tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Slides contained at least 70% disease-specific tissue. *KIT* (exons 9, 11 13, and 17) and *PDGFRA* (exons 12, 14, and 18) genes were amplified using the same primers and PCR conditions described elsewhere [12–14]. Briefly, DNA (100–200 ng) was amplified in a mixture containing 1× PCR buffer [20 mM TRIS (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$], dNTPs (200 mM each), primers (20 pM each), and 0.5 U Taq polymerase platinum (Invitrogen, Milan, Italy) in a 25 μl final volume. PCR conditions were: 8-min initial denaturation at 95 °C, then 35 cycles at 95 °C for 40 s, 55 °C for 40 s, and 72 °C for 40 s. After visualization onto agarose gel, PCR products were treated with ExoSAP-IT (USB Corp, Cleveland, OH) following the manufacturer's protocol, amplified with BigDye Terminator version 3.1 cycle sequencing kit (Applied Biosystems) using forward and reverse primers, and sequenced with an ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). Water was the negative control.

2.7. Ethics

The procedures followed were in accordance with the ethical standards of the local institutional committee on human