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ORIGINAL ARTICLE

Clinical Trials Study

Characterizing gastrointestinal stromal tumors and evaluating neoadjuvant imatinib by sequencing of endoscopic ultrasound-biopsies

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Author contributions: Hedenström P, Nilsson B, Nilsson O and Sadik R designed the research; Hedenström P, Nilsson B, Demir A, Andersson C, Enlund F, Nilsson O and Sadik R performed the research; Enlund F and Sadik R contributed new analytic tools; Hedenström P and Sadik R analyzed the data; Hedenström P wrote the paper.

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Abstract

AIM

To evaluate endoscopic ultrasound (EUS)-guided biopsies for the pretreatment characterization of gastrointestinal stromal tumors (GIST) to personalize the management of patients.

METHODS

All patients with lesions suspected to be GIST who were referred for EUS-sampling at a tertiary Swedish center were eligible for inclusion 2006-2015. During the observational study phase (2006-2011), routine fine-needle-aspiration (EUS-FNA) was performed.

In 2012-2015, we converted to an interventional, randomized protocol with dual sampling EUS-FNA and fine-needle-biopsy-sampling (EUS-FNB) for all lesions. c-KIT- and DOG-1-immunostaining was attempted in all samples and a manual count of the Ki-67-index was performed. FNB-sampled tissue and the resected specimens were subjected to Sanger sequencing of the *KIT* and platelet-derived growth factor alpha (*PDGFRA*) genes.

RESULTS

In all, 64 unique patients with GIST were included, and of these, 38 were subjected to pretreatment dual sampling. EUS-FNB had a higher diagnostic sensitivity when compared head-to-head with EUS-FNA (98% *vs* 58%, $P < 0.001$) and was more adequate for Ki-67-indexing (Ki-67$_{EUS}$) (92% *vs* 40%, $P < 0.001$). Sequencing of EUS-biopsies was successful in 43/44 (98%) patients, and the mutation profiles (*KIT*-mutation 73%, *PDGFRA*-mutation 18%, wild-type 7%) were fully congruent with those detected in the corresponding resected specimens. In imatinib-naïve patients, the Ki-67$_{EUS}$ was comparable with the Ki-67-index in the corresponding surgical specimens (Ki-67$_{SURG}$) (2.7% *vs* 2.9%, $P = 0.68$). In patients treated with neoadjuvant imatinib who also carried mutations indicating sensitivity, the Ki-67$_{EUS}$ was higher than the Ki-67$_{SURG}$ (2.5% *vs* 0.2%, $P = 0.005$), with a significant reduction in the Ki-67-index of -91.5% (95%CI: -82.4 to -96.0, $P = 0.005$).

CONCLUSION

EUS-guided biopsy sampling is accurate for the pretreatment diagnosis and characterization of GISTs and allows the prediction and evaluation of tumor response to neoadjuvant imatinib therapy.

Key words: Endosonography; Fine-needle biopsy; Gastrointestinal stromal tumor; *KIT*; Platelet-derived growth factor alpha; Tumor proliferation rate; Ki-67 index; Neoadjuvant treatment; Imatinib

Core tip: Personalization of the management and treatment of gastrointestinal stromal tumors (GIST) requires an extensive characterization of individual tumors. Information on the tumor proliferation rate and the *KIT*- and platelet-derived growth factor alpha (*PDGFRA*)-mutation profile is essential. While endoscopic ultrasound (EUS)-FNA is reported to be suboptimal for the diagnosis of GIST, EUS-guided biopsy sampling (EUS-FNB) has not been evaluated for the characterization of GISTs. This prospective, long-term study showed that EUS-FNB was safe and highly accurate for the pretreatment diagnosis of GISTs, for the sequencing of *KIT* and *PDGFRA*, and for the assessment of the tumor proliferation rate (Ki-67-index). By obtaining this information, we managed to guide and evaluate neoadjuvant imatinib therapy in patients with GIST.

Hedenström P, Nilsson B, Demir A, Andersson C, Enlund F, Nilsson O, Sadik R. Characterizing gastrointestinal stromal tumors and evaluating neoadjuvant imatinib by sequencing of endoscopic ultrasound-biopsies. *World J Gastroenterol* 2017; 23(32): 5925-5935 Available from: URL: http://www.wjgnet.com/1007-9327/full/v23/i32/5925.htm DOI: http://dx.doi.org/10.3748/wjg.v23.i32.5925

INTRODUCTION

In personalized medicine, a detailed characterization of tumors is essential for accurate patient management. A gastrointestinal stromal tumor (GIST) is an example of a tumor entity that illustrates the potential for genotype-driven targeted therapy[1]. However, to turn this potential into clinical reality, an extensive characterization of the tumor is needed.

First, GISTs are difficult to diagnose preoperatively. A sufficient quantity of tumor material is required for a conclusive diagnosis, which is reached by immunostaining for c-KIT (CD117), anoctamin 1 (DOG-1), or CD34[2].

Second, the tumor response to the tyrosine kinase inhibitor imatinib depends upon the mutation profile of the individual tumor. The genes commonly mutated in GIST are *KIT* proto-oncogene receptor tyrosine kinase (*KIT*) (exon 9, 11, 13 or 17), and less frequently, platelet-derived growth factor alpha (*PDGFRA*) (exon 12, 14 or 18). Primary resistance, or reduced sensitivity to imatinib, is related to mutations in exon 9, 13, or 17 of *KIT*, exon 18 of *PDGFRA*, or to the wild type profile (WT)[3]. Secondary resistance may evolve during imatinib treatment due to additional mutations[4]. Imatinib treatment of GISTs has led to a significant improvement in survival[5,6], and neoadjuvant imatinib is valuable, especially in advanced tumors[7,8]. The mutation profile is also a predictor of overall survival[9].

Third, the prognostic risk of GIST varies from excellent to poor[10]. In resected GIST specimens, the National Institutes of Health prognostic risk classification is used to assess the prognosis based on the tumor size and the tumor proliferation rate (the mitotic index [11]. The Ki-67-index is an alternative indicator of the tumor proliferation rate in GIST as well as in many other tumor entities. The level of the Ki-67-index in GIST strongly correlates with the prognosis[12-16].

Endoscopic ultrasound (EUS) enables the visualization of tumors such as GIST and the sampling with fine-needle aspiration (FNA) for cytology. The analysis of EUS-FNA-samples by mass spectrometry has been shown to facilitate the challenging assessment of cystic pancreatic lesions, a potential precursor of pancreatic adenocarcinoma[17]. In GISTs however, EUS-FNA-samples are often non-diagnostic[18-20], which also leads to an evident lack of prognostic information based on the tumor mutation profile and the tumor proliferation rate. This drawback of EUS-FNA is a major obstacle for the early personalized management of

patients with GIST. Confronted with the difficulties in the characterization of GISTs, clinicians have to decide on surgical resection based on the mere suspicion of malignancy and without knowledge of the tumor proliferation rate. Finally, the decision on expensive neoadjuvant imatinib treatment can only be based on probability and not on the actual mutation profile of *KIT* and *PDGFRA*.

The primary aim of the study was to evaluate EUS-guided sampling for the diagnosis and the pretreatment characterization of GIST with respect to the tumor proliferation rate and the mutation profile of *KIT* and *PDGFR*A. The secondary aim was to evaluate the Ki-67-index in EUS-biopsies and in resected specimens as a marker for individual tumor response to neoadjuvant imatinib therapy.

MATERIALS AND METHODS

Study patients

Sahlgrenska University Hospital is a tertiary center for advanced endoscopy and for the management of GIST in the region of west Sweden (population: 1.6 million). All patients who were referred to the unit for a diagnostic EUS-guided sampling of a suspicious GIST were eligible for inclusion in this prospective study as consecutive subjects. Findings suspected to be GIST were defined as lesions previously detected at gastroscopy or cross-sectional imaging with a probable origin from within the gastric or duodenal wall and with a hypoechoic appearance on ultrasound. Ongoing treatment with imatinib was a criterion for non-eligibility. Subjects were later excluded if the follow-up was consistent with an alternative diagnosis of the suspected lesion or if the GIST diagnosis could never be firmly established by conclusive histopathology including positive immunostaining for c-KIT or DOG-1.

The time frame for this study was February 2006 to December 2015. The medical records and the data from a parallel, prospective study on the long-term outcome of all patients with GIST in the region[15] were used to assess the results of EUS-guided sampling with respect to the clinical follow-up and the surgical outcome.

This study was reviewed and approved by the Regional Ethical Review Board of Gothenburg. Written informed consent was obtained from all patients.

This study is registered at ClinicalTrials. gov. The registration identification number is NCT02360839.

EUS - examination and sampling

All study subjects were examined by EUS under conscious sedation. Linear echoendoscopes [2006-2012: Pentax EG3830UT (Tokyo, Japan), 2012-: Pentax EG3870UTK] and an ultrasound processor (HI VISON Ascendus, Hitachi, Tokyo, Japan) were used for this purpose. The examinations were performed by the study endosonographer (RS). The tumor location, size, echogenicity, and vascularization were assessed before the optimal sampling route was chosen.

The Baseline Period (2006-2011): During the baseline period (BP), the study design was observational. All patients were prospectively included except for the first nine patients of the study start-up phase.

The suspicious GISTs were sampled at the discretion of the endosonographer with no specific interventional procedure. EUS-FNA was performed with either a 22 G or a 25 G needle (Olympus, Aomori, Japan/Boston Scientific, Spencer, United States/Wilson-Cook Medical, Limerick, Ireland), while a 19 G trucut-needle (TCB) was used (Wilson-Cook Medical) for biopsies.

The Study Period (2012-2015): We designed an interventional study protocol in 2011 and modified the sampling procedure in 2012. From 2012 to 2015 (Study Period, SP), dual sampling was performed on *each* individual subject using both EUS-FNA for cytology (needles as described above) and EUS-guided core biopsy sampling (EUS-FNB) for histology (22 G Procore or 19 G Procore, Wilson-Cook Medical)[21]. In blocks of four and by using sealed envelopes, the patients were randomized to a first pass with FNA or FNB. This was performed to eliminate the introduction of a bias related to the sampling sequence. Further passes were performed by alternating the needles. A non-necrotic area of the tumors was targeted and sampling was performed by fanning. If the yield was poor, the sampling time and the suction were increased.

The first six subjects of the *Study Period* underwent EUS-FNB only to accustom the endosonographer to the new sampling technique. With some limitations, a cytotechnician was present for rapid on-site cytology evaluation.

Cytopathology and histopathology

FNA-samples and FNB-biopsies were processed and analyzed as described in the Supplementary Methods.

Ki-67-indexing

Representative samples were subjected to immunostaining for Ki-67 as described in the Supplementary Methods. The quality and the adequacy of the FNA-samples and the FNB-biopsies for the assessment of the Ki-67-index were categorized as adequate or non-adequate by the study cytopathologist (AD) and pathologist (ON).

Given the superior quality of the FNB-biopsies compared with FNA-samples, only the Ki-67-index of FNB-biopsies (Ki-67$_{EUS}$) was calculated in detail on printouts of digital images captured *via* an x40-magnification objective (Eclipse E1000, Nikon, Japan) with a ProgResC7-camera (Jenoptik, Germany). Manual counting of positive nuclei including 2000 tumor cells was performed. Counting by eyeballing and digital

counting are considered less accurate and were not used[22]. The result was recorded as the fraction of positive tumor cells (%). Similarly, the Ki-67-index of the corresponding surgical specimens ($Ki\text{-}67_{SURG}$) was analyzed in subjects who underwent resection.

In each case sampled by EUS-FNB *and* subjected to surgical resection, we calculated the following parameters: (1) The pairwise difference in the Ki-67-index (%-units): $Ki\text{-}67_{DIFF} = Ki\text{-}67_{EUS} - Ki\text{-}67_{SURG}$; and (2) The pairwise reduction in the Ki-67-index (%): $Ki\text{-}67_{RED} = -100 \times [1-(Ki\text{-}67SURG)/(Ki\text{-}67EUS)]$

Sequencing and mutational analysis

No sequencing of FNA-samples was performed since the sample quantity and quality were poor compared with that of FNB-biopsies. All FNB-biopsies were subjected to mutational analysis by Sanger sequencing as were the corresponding resected specimens (in subjects who underwent resection). In the early part of the SP, the sequencing of FNB-biopsies was performed for research purposes after EUS. In the latter part of the SP, the procedure was implemented into clinical practice and was performed directly after EUS to supply the genetic information to the clinician (BN).

The preparation of FNB-biopsies for DNA-extraction followed by sequencing is described in the Supplementary Methods.

Follow-up, reference standard, and definitions

Subjects were followed-up by the clinician (BN) for 5 year or until death. Neoadjuvant imatinib therapy was considered and initiated by the clinician (BN). Patients having small tumors (size < 20 mm) were not evaluated for neoadjuvant imatinib. The cases subjected to surgical resection, either treated or not treated with neoadjuvant imatinib, were designated as: (1) Neo- (no neoadjuvant imatinib therapy); (2) Neo + s (neoadjuvant imatinib and imatinib-sensitive mutation profile); or (3) Neo + r (neoadjuvant imatinib therapy and imatinib-resistant mutation profile) according to the table in the Supplementary Methods. The tumor response was evaluated on a clinical basis in some cases *via* the comparison of the fluorodeoxyglucose positron emission tomography (^{18}FDG-PET) signal at baseline and at 3-8 wk after the start of imatinib treatment.

Resected specimens were used to validate the diagnosis of GIST. In patients not subjected to surgery, the GIST-diagnosis was considered established if cytopathology or histopathology of tumor sampling was conclusive for GIST including positive immunostaining for KIT or DOG-1.

The FNA-samples and FNB-biopsies were classified as diagnostic only if they contained adequate GIST material for accurate diagnostic KIT or DOG-1 immunostaining. Samples with adequate tumor yield but with failed or inconclusive immunostaining were classified as suggestive of GIST. Samples without adequate tumor yield were considered non-diagnostic.

Outcome

The primary outcome of this study was the diagnostic sensitivity of EUS-guided sampling for GIST. The secondary outcome was the EUS-sample adequacy (1) for the assessment of the Ki-67-index; (2) for the sequencing of *KIT* and *PDGFRA* (FNB-biopsies only); and (3) for the evaluation of response to neoadjuvant imatinib therapy (FNB-biopsies only), which was measured as the difference in the Ki-67-index of FNB-biopsies compared with that of resected specimens.

Statistical analysis

Demographics, tumor characteristics, and procedures were compared using Fisher's exact test and the Mann-Whitney *U*-test. Prior to the interventional phase (the SP), a sample size calculation was performed for paired, dichotomous variables (statistical power = 80%, alpha error = 0.05), which aimed to detect a difference in sensitivity of 35% in order to compare EUS-FNA and EUS-FNB at dual sampling. A sample size of 33 cases was returned.

The diagnostic sensitivity for GIST as a binary outcome was compared between sampling groups using Fisher's exact test (unpaired data) and McNemar's test (paired data) in an intention-to-treat analysis. The Ki-67-index of FNB-biopsies and resected specimens was compared using the Wilcoxon signed-rank test. The mutation profile of FNB-biopsies and resected specimens was compared on a case-by-case basis. The (95%CI) was calculated when possible. The statistical significance level was set at $P < 0.05$. All authors had access to the study data and approved the manuscript. The STARD protocol was applied throughout the study.

RESULTS

Patient characteristics

In total, 64 patients [34 women/30 men, median age 70 (range: 23-89)] were included (Figure 1). Validation specimens were available in 43/64 (67%) cases (resected specimen: 42 cases, endoscopy forceps: one case). The baseline characteristics are shown in Table 1.

Primary outcome

The diagnostic sensitivity of EUS-FNB (dual procedures FNB+FNA: $n = 38$, single FNB-procedures: $n = 6$) was superior both compared with routine EUS-FNA performed during the *Baseline Period* [43/44 (98%) *vs* 8/16 (50%), $^{a}P < 0.001$] and compared with EUS-FNA in a head-to-head comparison of dual sampling procedures during the SP [37/38 (97%) *vs* 22/38 (58%), $^{b}P < 0.001$], as shown in Figure 2.

Supposing that the two cases in Figure 1, which had an unclear final diagnosis during the *Study Period*, were actually true GISTs, the worst scenario of the

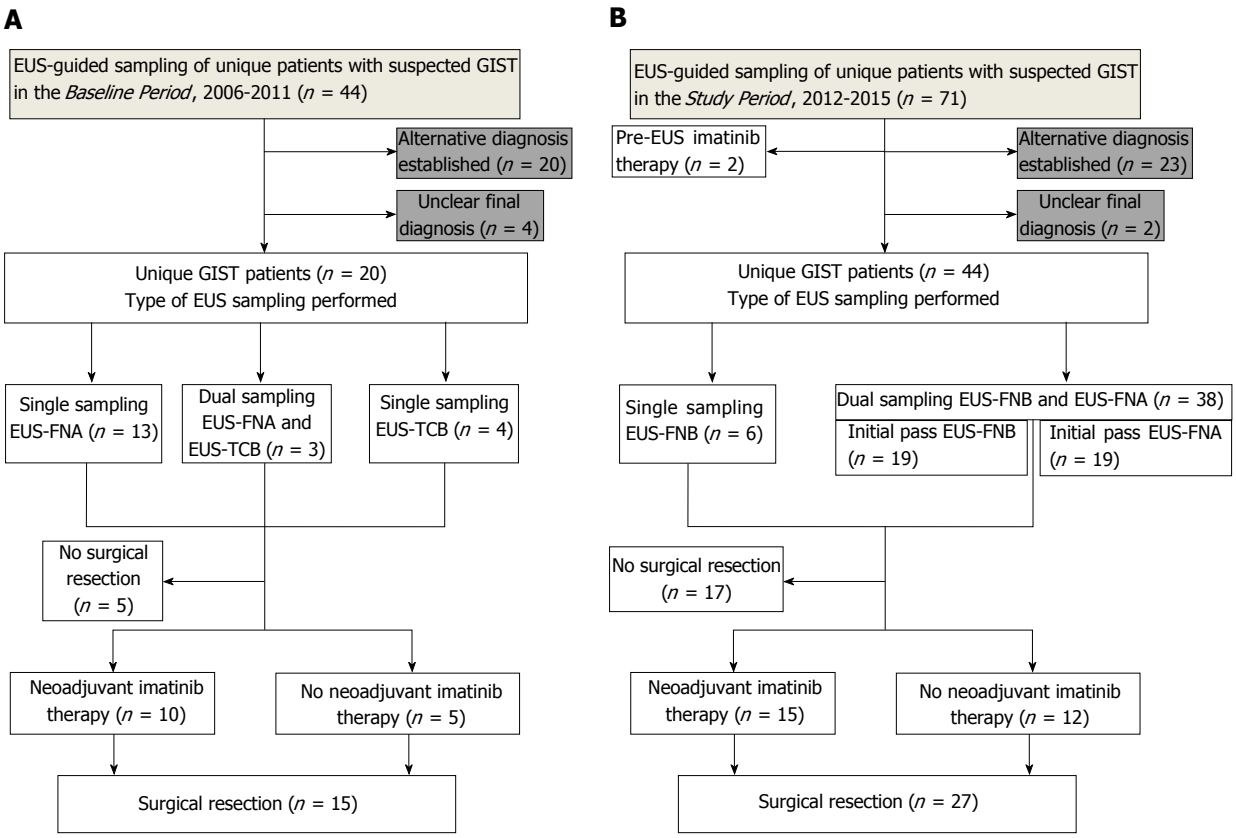

Figure 1 Flow charts of the study inclusion process, February 2006-December 2015. A: The *Baseline Period*, observational study design (2006-2011); B: The *Study Period*, interventional study design (2012-2015). GIST: Gastrointestinal stromal tumor; EUS: Endoscopic ultrasound; FNA: Fine-needle aspiration; FNB: Fine-needle biopsy.

Table 1 Baseline characteristics, follow-up, and clinical outcome *n* (%)

Parameter	Baseline period	Study period	*P* value
Age, median (range)	75 (23-89)	68 (49-89)	0.07
Gender (M/F)	11/9	19/25	0.43
Study patients (*n*)	20	44	
Tumor location (*n*)			
Stomach	18	40	
Duodenum	2	4	
Tumor size (mm), median (range)	60 (12-200)	38 (13-220)	0.29
Tumor endosonographic appearance			
Homogenous (solid)	8	17	
Heterogeneous (necrotic)	12	27	
EUS-FNA (*n*)	16	38	
Needle (22 G/25 G)	12/4	26/12	0.75
Passes (*n*), median (range)	2 (1-3)	3 (1-4)	0.10
EUS-FNB (*n*)	7	44	
Needle (TCB 19 G/FNB 19 G/FNB 22 G)	7/0/0	0/5/39	< 0.001
Passes (*n*), median (range)	1 (1-4)	2 (1-4)	0.15
ROSE[1]	9 (56)	26 (68)	0.53
Study cytologist	5 (31)	32 (84)	< 0.001
Study pathologist	2 (29)	38 (86)	0.003
Resected cases	15 (75)	27 (61)	0.40
Resection margin (R0/R1/R2)	13/1/1	24/3/0	
Follow-up time[2], mo (range)	72 (16-105)	19 (1-45)	
Overall survival (OS)[3], 12 mo	20/20 (100)	31/31 (100)	1.00
OS, 24 mo	19/20 (95)	17/18 (94)	1.00
OS, 36 mo	18/20 (90)	10/11 (91)	1.00
Patients deceased	9/20 (45)	2/44 (5)	

[1]Rapid on-site cytology evaluation by a cytotechnician; [2]From the date of EUS until death or until end of follow-up; [3]From the date of EUS until 12, 24 and 36 mo post-EUS. EUS: Endoscopic ultrasound; FNA: Fine needle aspiration; FNB: Fine needle biopsy.

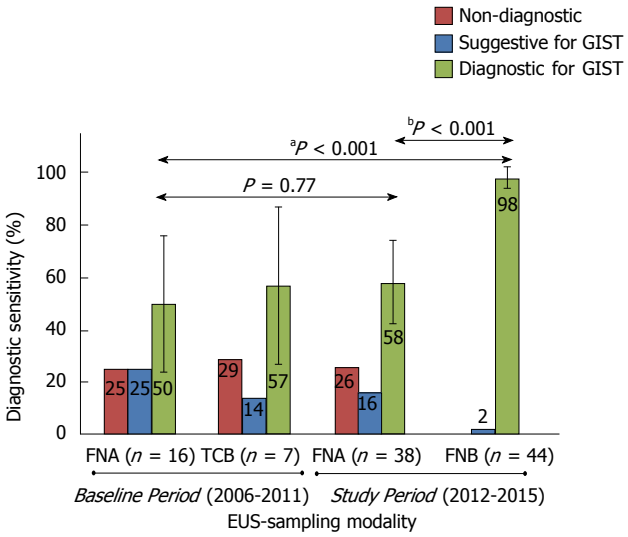

Figure 2 The diagnostic sensitivity of endoscopic ultrasound-guided sampling in unique Gastrointestinal stromal tumor-cases (n_{tot} = 64) examined at the Sahlgrenska University Hospital from 2006 to 2015. The error bars represent the 95%CI. FNA: Fine-needle aspiration; FNB: Fine-needle biopsy; TCB: Trucut-biopsy; GIST: Gastrointestinal stromal tumor; EUS: Endoscopic ultrasound.

sensitivity of EUS-FNB would still be superior to that of EUS-FNA in a head-to-head comparison (37/40, 93% *vs* 22/40, 55%, $P < 0.001$). The sensitivity of FNA-samples was not affected by the recorded variables, as shown in Table 2.

One minor adverse event was recorded (1/64, complication rate 1.6%). Patient *#33*, who had a 3-cm GIST in the stomach, experienced local bleeding post-EUS, which was stopped by adrenalin injection. No technical failure was observed for any needle. No tumor seeding was observed in any of the patients during follow-up.

Secondary outcomes

Ki-67-indexing: During the BP, the FNA-samples were of adequate quality for the assessment of the Ki-67-index in 3/16 (19%) cases. In the dual sampling procedures during the SP, FNB-biopsies were more often of adequate quality (37/38, 92%) compared with FNA-samples (15/38, 40%, $P < 0.001$), and the FNB-biopsies were adequate for the assessment of the Ki-67-index in all cases subjected to surgical resection 27/27 (100%).

In non-resected cases with adequate FNB-biopsies (n = 14), the mean Ki-67$_{EUS}$ was 6.1% (95%CI: 2.5 to 9.7).

In resected cases not treated with neoadjuvant imatinib (n = 12, Neo- Group), the median Ki-67$_{EUS}$ was not significantly different from the median Ki-67$_{SURG}$ [2.7% *vs* 2.9%, ^aP = 0.68, median Ki-67$_{DIFF}$ = -0.30 (95CI: -0.62 to 0.57, P = 0.64)] (Figures 3A, 4A and B). No significant reduction was observed in the Ki-67-index [median Ki-67$_{RED}$ = 10.7% (95%CI: -22.3 to 26.5, P = 0.70)].

Table 2 Parameters with potential influence on the sensitivity of Endoscopic ultrasound-fine needle aspiration *n* (%)

Parameter			*P* value
Tumor echogenicity	Homogenous (solid)	Heterogeneous (necrotic)	
EUS-FNA-sensitivity	11/20 (55)	18/32 (56)	1.0
Tumor size	< 30 mm	⩾ 30 mm	
EUS-FNA-sensitivity	10/18 (56)	19/34 (56)	1.0
ROSE	ROSE	non-ROSE	
EUS-FNA-sensitivity	21/34 (62)	8/18 (44)	0.26
FNA-needle	22 gauge	25 gauge	
EUS-FNA-sensitivity	20/37 (54)	9/15 (60)	0.77
FNA-passes	< 3 passes	⩾ 3 passes	
EUS-FNA-sensitivity	9/17 (53)	20/35 (57)	1.0
Sampling order[1]	EUS-FNA first	EUS-FNB first	
EUS-FNA-sensitivity	12/19 (61)	10/19 (53)	0.63

[1]Only the GISTs (n = 38) sampled during the Study Period (2012-2015). All study GISTs examined by EUS-FNA (n = 52) from 2006 to 2015. GIST: Gastrointestinal stromal tumor; EUS: Endoscopic ultrasound; FNA: Fine needle aspiration; FNB: Fine needle biopsy.

Sequencing of *KIT* and *PDGFRA*: The FNB-biopsies were adequate for successful Sanger sequencing of *KIT* and *PDGFRA* in 43/44 (98%) cases (Table 3). Among resected cases, full congruence (100%) was found in the comparison of the mutations detected in the FNB-biopsies and the mutations detected in the corresponding resected specimens (n = 27). Additional mutations in *KIT* or *PDGFRA* were not observed in any of the resected specimens. The sole FNB-biopsy (case *#2*) with inadequate material for diagnostic immunohistochemistry still contained sufficient material for successful sequencing.

Evaluation of neoadjuvant imatinib therapy: (1) Neoadjuvant imatinib + imatinib-sensitive mutation detected (Neo + s Group): In resected patients who were treated with neoadjuvant imatinib and who carried a mutation suggestive of primary sensitivity to imatinib [n = 10: *KIT* exon 11 (n = 9); *PDGFRA* exon 12 (n = 1)], the median Ki-67$_{EUS}$ was significantly higher than the median Ki-67$_{SURG}$ [2.5% *vs* 0.2%, P = 0.005, median Ki-67$_{DIFF}$ = 2.3 (95%CI: 0.67 to 5.37, P = 0.005)] (Figures 3B, 4C and D). Consequently, a significant reduction was observed in the Ki-67-index [median Ki-67$_{RED}$ = -91.5% (95%CI: -82.4 to -96.0, P = 0.005)].

In the five patients with a positive baseline ^{18}FDG-PET, a signal reduction was recorded in the post-treatment ^{18}FDG-PET signal.

(2) Neoadjuvant imatinib + imatinib-resistant mutation detected (Neo + r Group): Five resected patients who were treated with neoadjuvant imatinib carried a mutation suggestive of primary resistance to imatinib [n = 5: *PDGFRA* exon 18 D842V (n = 2); WT (n = 2); *KIT* exon 13 p. K642E (n = 1)]. The median Ki-67$_{EUS}$

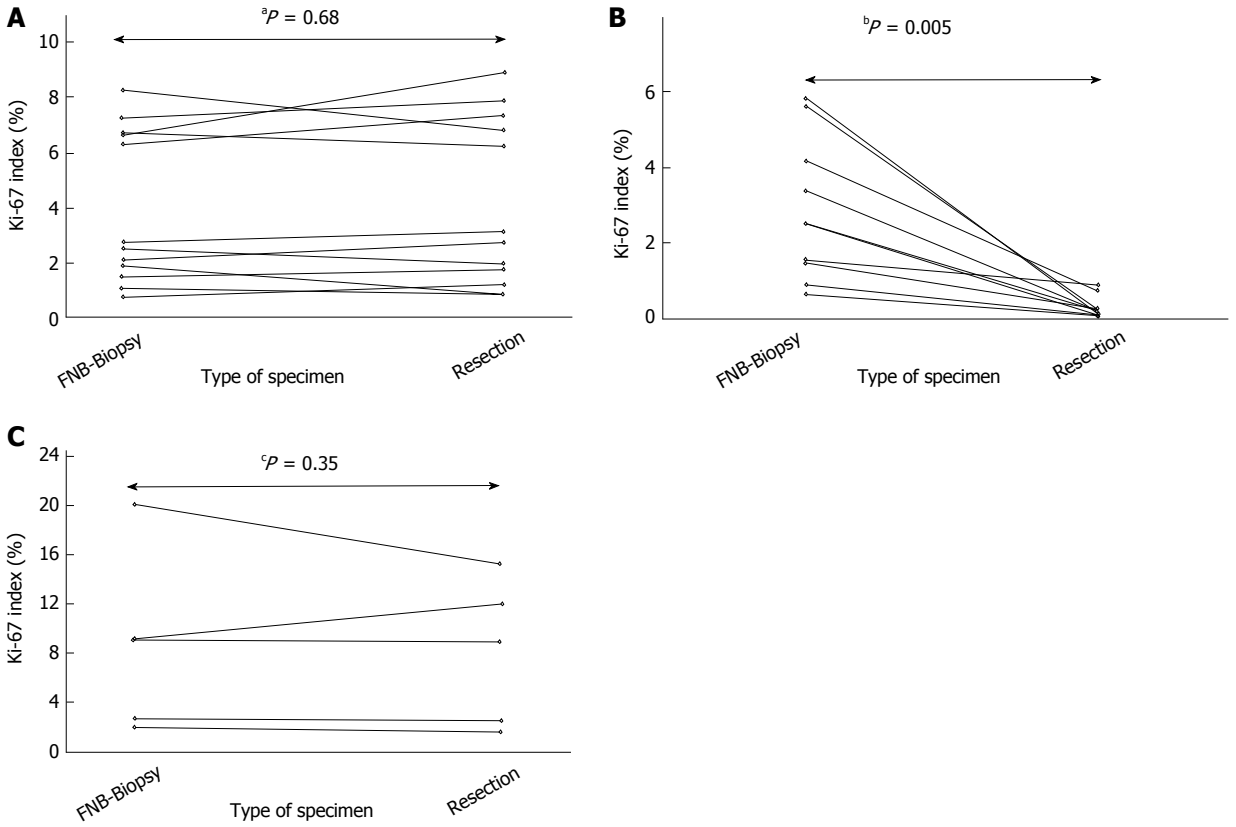

Figure 3 The Ki-67-index (%) of the fine-needle biopsy and of the corresponding resected specimen in each patient who underwent resection. A: Patients not treated with neoadjuvant imatinib; B: Patients treated with neoadjuvant imatinib who carried an imatinib-sensitizing *KIT*- or *PDGFRA*-mutation; C: Patients treated with neoadjuvant imatinib who carried a *KIT*- or *PDGFRA*-mutation (or wild type profile), which indicates resistance to imatinib.

was not significantly different from the median Ki-67$_{SURG}$ (9.1% *vs* 9.0%, P = 0.35) (Figures 3C, 4E and F). In addition, no significant reduction was observed in the Ki-67-index (median Ki-67$_{RED}$ = -10.2%, P = 0.50). The baseline ^{18}FDG-PET signal was measured and was positive in two patients. In one patient, no reduction was observed in the post-treatment ^{18}FDG PET-signal, while a weak reduction was recorded in the other (case *#20*).

DISCUSSION

This study provides new knowledge on the ability to perform extensive preoperative and pretreatment characterization of gastrointestinal stromal tumors. This knowledge enables the introduction of an early personalized management and treatment of patients with GIST.

According to the results of this work, endoscopic ultrasound-guided biopsy sampling is a safe and accurate method for the purpose of diagnosis and for further analyses of the tumor material in GIST.

A correct and reliable diagnosis of GIST is important to avoid unnecessary resections of benign lesions that are merely suspected GISTs. A non-diagnostic sample will result in uncertain management and a resection based on suspicion alone. Prospective studies that evaluate the accuracy of EUS-guided sampling of GIST are scarce. Studies have reported a diagnostic sensitivity of approximately 50%[18], which is in agreement with the sensitivity of EUS-FNA in our work. A sensitivity of 80% was reported in a recent study that excluded small tumors (< 20 mm)[23].

In the present study, a new method of dual sampling with both EUS-FNA and EUS-FNB was used on all tumors during a 4-year period, and these modalities were compared head-to-head. As a result, we have now shown that EUS-FNB can be used for the reliable and safe diagnosis of GIST in up to 98% of cases including small tumors.

The treatment decision for GIST requires a balance between the benefits and drawbacks of both surgical and pharmacological therapies. The initiation of adjuvant and neoadjuvant treatment can vary in between institutions and the prognostic risk needs to be addressed since there are potential side-effects of imatinib. Nevertheless, the treatment with tyrosine kinase inhibitors should be prescribed only to patients who carry sensitive mutations. Without the mutational status of *KIT* and *PDGRFA*, the clinician randomly selects the therapy. The mutation profile is also valuable for the prediction of survival[9].

This study shows that a mutational analysis of pretreatment FNB-biopsies by Sanger sequencing provides the genetic information needed. In the early part of the SP the clinician had to decide on

Table 3 Individual case data on the mutation profile, treatment, and Ki-67-index

Case	Mutation gene and exon	Mutation	Treatment neoadj[1]	Surgery	Group	EUS-surgery (mo)	Ki67EUS	Ki67SURG	Ki67RED
1	*KIT* exon 11	p.V560del	No	Yes	Neo-	2	2.2	2.8	26
2	*PDGFRA* exon 18	p.D842V[2]	No	No	NA	-	NC	-	-
3	Wild Type	Wild Type	Yes	Yes	Neo + r	2	2.1	1.7	-19
4	*KIT* exon 11	p.V559D	Yes	Yes	Neo + s	6	4.2	0.7	-82
5	*KIT* exon 11	p.Y553-Q556del	Yes	Yes	Neo + s	13	1.5	0.2	-84
6	Unknown	Unknown	No	No	NA	-	NC	-	-
7	*KIT* exon 11	V559D	No	No	NA	-	2.4	-	-
8	*KIT* exon 11	p.P577-R586dupl	No	Yes	Neo-	2	6.3	7.4	17
9	*KIT* exon 11	V559del	Yes	Yes	Neo + s	9	2.5	0.1	-96
10	*KIT* exon 11	p.V560D	No	Yes	Neo-	1	1.5	1.8	17
11	*KIT* exon 11	p.V560D	No	Yes	Neo-	2	0.8	1.2	47
12	*KIT* exon 11	p.V560del	Yes	No	NA	-	19.3	-	-
13	*KIT* exon 11	p.V559D	No	Yes	Neo-	3	1.9	0.9	-52
14	*KIT* exon 11	V559G	Yes	Yes	Neo + s	16	1.6	0.9	-43
15	*PDGFRA* exon 18	p.D842V	Yes	Yes	Neo + r	2	9.1	9.0	-1
16	*KIT* exon 11	p.W557G	Yes	Yes	Neo + s	12	0.6	0.1	-93
17	*KIT* exon 11	p551-W557delinsR	Yes	Yes	Neo + s	12	3.4	0.2	-94
18	*KIT* exon 11	D579del	No	Yes	Neo-	2	6.7	6.3	-6
19	*PDGFRA* exon 12	E556-I565dupl	Yes	Yes	Neo + s	2	0.9	0.1	-89
20	*KIT* exon 13	p K642E	Yes	Yes	Neo + r	4	20.1	15.3	-24
21	*KIT* exon 11	p.W557R	No	Yes	Neo-	2	7.2	7.8	9
22	*KIT* exon 11	V559D	Yes	Yes	Neo + s	2	5.6	0.3	-95
23	*KIT* exon 11	P551-E554delinsQ	Yes	Yes	Neo + s	12	5.8	0.1	-98
24	*KIT* exon 11	K558-G565delinsR	Yes	No	NA	-	21.5	-	-
25	*PDGFRA* exon 18	p.D842V	No	No	NA	-	1.5	-	-
26	*PDGFRA* exon 18	p.D842V	Yes	Yes	Neo + r	1	2.7	2.5	-10
27	*KIT* exon 11	V559D	No	Yes	Neo-	3	2.8	3.1	13
28	*KIT* exon 11	p.V559D	No	Yes	Neo-	2	1.1	0.8	-25
29	*KIT* exon 11	p.V559D	Yes	Yes	Neo + s	17	2.5	0.2	-90
30	*KIT* exon 11 *KIT* exon 13	pQ575-L576dupl V654A	No	No	NA	-	2.7	-	-
31	*KIT* exon 11	V559D	No	No	NA	-	1.4	-	-
32	*KIT* exon 11	p.L576P	No	No	NA	-	1.8	-	-
33	*PDGFRA* exon 18	p.D842V	No	Yes	Neo-	2	6.6	8.9	35
34	*PDGFRA* exon 18	p.D846Y	No	Yes	Neo-	2	2.6	2.0	-22
35	*KIT* exon 11	p P551-W560del	No	No	NA	-	3.0	-	-
36	*KIT* exon 11	V560E	Yes	No	NA	-	NC	-	-
37	Wild type	Wild type	Yes	Yes	Neo + r	3	9.2	12.0	31
38	*KIT* exon 11	pL567del	Yes	No	NA	-	10.1	-	-
39	*PDGFRA* exon 12	M578-S584del	Yes	No	NA	-	11.0	-	-
40	*KIT* exon 11	p.P551-Q556del	Yes	No	NA	-	4.6	-	-
41	*KIT* exon 11	p.N567-T574del	No	Yes	Neo-	1	8.3	6.8	-18
42	*KIT* exon 11	57-E561del	Yes	No	NA	-	4.6	-	-
43	Wild type	Wild type	No	No	NA	-	0.1	-	-
44	*KIT* exon 9	A502-Y503dupl	Yes	No	NA	-	0.7	-	-

[1]Neoadjuvant treatment with imatinib; [2]For comparison, the sequencing was performed on an endoscopy biopsy. The 44 GIST study cases sampled with EUS-FNB and ordered by the date of study enrollment. Ki-67$_{EUS}$: The Ki-67$_{index}$ (%) of the EUS-biopsies; Ki-67$_{SURG}$: The Ki-67$_{index}$ (%) of the resected specimens; Ki-67$_{RED}$: The percentage of the reduction in the Ki-67$_{index}$, comparison of the Ki-67$_{EUS}$ with the Ki-67$_{SURG}$ (see Methods); NA: Not annotated; NC: Not countable; *KIT*: KIT proto-oncogene receptor tyrosine kinase; *PDGFRA*: Platelet-derived growth factor receptor alpha.

neoadjuvant imatinib therapy without information on the mutational status. Consequently, in five patients who were treated with neoadjuvant imatinib, the sequencing of FNB-biopsies later showed a genetic profile consistent with primary resistance to imatinib, which led to a modification in the treatment regimen. To assist clinicians during the preoperative management of patients with GIST, we implemented the immediate sequencing of FNB-biopsies during the latter part of the SP. One recent retrospective study revealed that it is possible to obtain the mutation profile of GISTs in a selected pool of EUS-FNA-samples using next-generation sequencing[24]. No comparison was made between the sequencing of EUS-FNA-samples and the sequencing of any corresponding resected specimens.

The prognosis of an individual patient with GIST is dependent on the tumor proliferation rate and the size of the tumor[15,25]. In our study, the FNB-biopsies were highly accurate for the precise assessment of the Ki-67-index by manual counting. The Ki-67-index measured in the FNB-biopsies seems reliable since it was in agreement with the Ki-67-index of the resected specimens of the study patients who were not treated with neoadjuvant imatinib. More

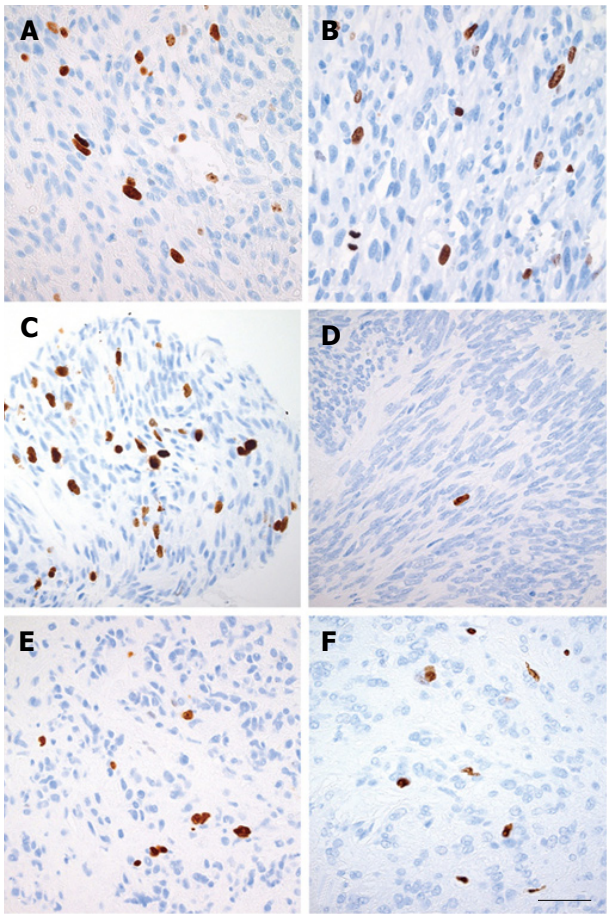

Figure 4 Ki-67-immunostaining of gastrointestinal stromal tumors-tumor tissue in three endoscopic ultrasound-biopsies and in the three corresponding resected specimens. Digital photos (magnification × 40): EUS-biopsy-tissue (left) and resected specimen tissue (right). Cell nuclei (brown color) are positive for Ki-67 while other cell nuclei (blue color) are negative for Ki-67. Scale bar equals 50 μm. A and B: Case #18, *Neo*- group (*KIT* exon 11 D579del, Ki-67$_{EUS}$: 6.6%, Ki-67$_{SURG}$: 6.3%); C and D: Case #22, *Neo* + s group (*KIT* exon 11 V559D, Ki-67$_{EUS}$: 5.6%, Ki-67$_{SURG}$ 0.3%); E and F: Case #26, *Neo* + r group (*PDGFRA* exon 18 p.D842V, Ki-67$_{EUS}$: 2.7%, Ki-67$_{SURG}$ 2.5%). EUS: Endoscopic ultrasound.

importantly, in patients who are sensitive to imatinib and who are treated with neoadjuvant imatinib, the Ki-67-index of FNB-biopsies probably better reflects the accurate proliferation rate of tumors compared with the Ki-67-index of resected specimens, which may erroneously be found to be low. A substantial danger of the overestimation of survival and the under-prescription of adjuvant therapy can emerge in these groups of patients. An assessment of the mitotic rate of specimens obtained by FNB-biopsy is probably challenging, and it was not an aim of this study. The maximum quantity of FNB-material obtained in this study reached 40 high-power fields.

The pretreatment assessment of the Ki-67-index has a range of clinical applications. This assessment provides clinicians with prognostic information for a discussion of therapeutic options with their patients. The tumor response to neoadjuvant treatment by measurement of the reduction in the Ki-67-index, as described in the current study, may guide adjuvant treatment in patients who undergo resection. ^{18}FDG-PET is expensive and some tumors may have a negative baseline signal; the demonstration of the Ki-67-indexing of repeated EUS-biopsies is an attractive method by which the therapeutic response may be evaluated.

This prospective study was conducted in a large Swedish region over several years and involved dedicated experts and the use of advanced techniques. The centralized management of GIST facilitated good control of patients and reliable follow-up data. We used pretreatment tumor tissue not only to diagnose GIST but also to clarify the sensitivity to imatinib, to assess the tumor proliferation rate, and finally, to evaluate the treatment response to imatinib. To the best of our knowledge, the presented results are more detailed and accurate than those of any comparable publications in the literature.

A limitation of EUS is that GISTs in the jejunum or ileum can be punctured only if they are visible from the stomach or the duodenum. However, the majority of GISTs are located in the stomach. Some study patients were treated with neoadjuvant imatinib even if they carried mutations with primary resistance to imatinib, which highlights the importance of sequencing prior to therapy. Sampling errors may result in an erroneously low Ki-67-index. However, such a phenomenon was probable only in two patients in this study (case *#33* and *#37*).

The described pretreatment characterization of tumors should be incorporated in future management guidelines of GIST to facilitate personalized treatment. Moreover, the work-up of complex tumors such as GISTs should be centralized to high-volume centers in order to enable a rational and effective treatment.

We conclude that this study provides clear support for endoscopic ultrasound as the front-line diagnostic procedure in GIST, as it enables an early diagnosis and a personalized, genotype-driven targeted therapy of patients. The presented approach with the extensive characterization of GISTs based on the analysis of EUS-guided biopsies may also serve as a model for other tumor entities.

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COMMENTS

Background

The early personalized management and treatment of gastrointestinal stromal tumors (GISTs) require an extensive characterization of individual tumors. Information on the tumor proliferation rate and the *KIT*- and platelet-derived

growth factor alpha-mutation profile is essential.

Research frontiers

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) has been reported to be suboptimal for the diagnosis of GIST, but endoscopic ultrasound (EUS)-guided biopsy sampling (EUS-FNB) has not been evaluated for the characterization of GISTs. Neither the Ki-67 index nor KIT/PDGFRA-sequencing has been evaluated in EUS-FNB-tissue.

Innovations and breakthroughs

This prospective, long-term study showed that EUS-FNB was safe and highly accurate for the pretreatment diagnosis of GISTs, for the sequencing of *KIT* and *PDGFRA*, and for the assessment of the tumor proliferation rate (Ki-67-index). To the best of our knowledge, other relevant publications in this field demonstrate a diagnostic accuracy of EUS-FNA of approximately 50%. The sequencing of EUS-FNA-smears of GIST has not been evaluated in a prospective cohort but only in a single, retrospective study that included 20 patients.

Applications

By obtaining the extensive, preoperative diagnostic and prognostic information described in the present study, it will be possible to personalize the clinical and surgical management of patients with GIST especially with respect to the guidance and evaluation of neoadjuvant imatinib therapy.

Peer-review

This manuscript is about endoscopic ultrasound-guided biopsy in the diagnosis of gastrointestinal stromal tumors and evaluating neoadjuvant imatinib by sequencing of EUS-biopsies. It's an interesting and valuable manuscript.

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