Original article

Genotype and risk of tumour rupture in gastrointestinal stromal tumour

K. Boye[1,2], J.-M. Berner[3], I. Hompland[1,5], Ø. S. Bruland[1,5], S. Stoldt[4], K. Sundby Hall[1], B. Bjerkehagen[3,6] and T. Hølmebakk[4]

[1]Department of Oncology and [2]Department of Tumour Biology, Institute for Cancer Research, and [3]Department of Pathology, and [4]Department of Abdominal and Paediatric Surgery, Oslo University Hospital, The Norwegian Radium Hospital, and [5]Institute of Clinical Medicine [6]Department of Oral Biology, University of Oslo, Oslo, Norway

Correspondence to: Dr K. Boye, Department of Oncology, Oslo University Hospital, The Norwegian Radium Hospital, PO Box 4953 Nydalen, NO-0424 Oslo, Norway (e-mail: kjetil.boye@rr-research.no; @kjetilboye, @Oslounivsykehus)

Background: Tumour rupture is a strong predictor of poor outcome in gastrointestinal stromal tumours (GISTs) of the stomach and small intestine. The objective was to determine whether tumour genotype was associated with risk of rupture.

Methods: Rupture was classified according to the definition proposed by the Oslo Sarcoma Group. Since January 2000, data were registered retrospectively for all patients at Oslo University Hospital undergoing surgery for localized GIST of the stomach or small intestine. Tumour genotype was analysed by Sanger sequencing.

Results: Two hundred and nine patients with mutation data available were identified. Tumour rupture occurred in 37 patients. Among the 155 patients with *KIT* exon 11 mutations, an increased risk of rupture was observed with a deletion or insertion–deletion (25 of 86, 29 per cent) compared with substitutions (5 of 50, 10 per cent) or duplications/insertions (2 of 19, 11 per cent) (P = 0·014). Notably, rupture occurred in 17 of 46 tumours (37 per cent) with deletions involving codons 557 and 558 (del557/558) *versus* 15 of 109 (13·8 per cent) with other exon 11 mutations (P = 0·002). This association was confined to gastric tumours: 12 of 34 (35 per cent) with del557/558 ruptured *versus* six of 77 (8 per cent) with other exon 11 mutations (P = 0·001). In multivariable logistic regression analysis, del557/558 and tumour size were associated with an increased likelihood of tumour rupture, but mitotic count was not.

Conclusion: Gastric GISTs with *KIT* exon 11 deletions involving codons 557 and 558 are at increased risk of tumour rupture. This high-risk feature can be identified in the diagnostic evaluation and should be included in the assessment when neoadjuvant imatinib treatment is considered.

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Introduction

Tumour rupture increases the risk of recurrence of gastrointestinal stromal tumours (GISTs) of the stomach and small intestine[1–4]. Rupture may occur spontaneously and before diagnosis, or during surgery owing to manipulation of the tumour. The reported incidence of tumour rupture in GIST varies considerably, ranging from 2 to 22 per cent in different series[4–8]. Until recently, there has been no precise definition of rupture, possibly explaining these divergent results. Using a strict definition of tumour rupture, the sarcoma group at Oslo University Hospital (OUH) has demonstrated that rupture is a strong and independent risk factor for recurrence, also when other established prognostic factors are taken into consideration[2].

Large tumour size, high mitotic count and tumour location in the small intestine are all associated with an increased risk of rupture[2]; of these, only size and location are known before surgery. Improved prediction of patients at risk might influence therapeutic decisions. According to current guidelines, preoperative treatment with the tyrosine kinase inhibitor imatinib could be considered if the surgery is then expected to be safer[9]. There are, however, no clearly established criteria for selection of patients for such treatment.

Mutations in the *KIT* or *PDGFRA* (platelet-derived growth factor α) gene are found in the majority of

GISTs[10–12]. Certain mutations are associated with a more aggressive phenotype; patients with deletions involving codons 557 and 558 in *KIT* exon 11 have a particularly poor outcome[13–15]. The present study investigated whether tumour genotype is associated with tumour rupture using the definition of rupture proposed by the Oslo Sarcoma Group.

Methods

Patients undergoing surgery for GIST of the stomach or the small intestine from January 2000 to April 2017 were identified in the clinical sarcoma database of OUH, which contains prospective data on patients treated for bone or soft tissue sarcoma within the South-East Health Region of Norway, with a population of 2·8 million. Patients with metastatic disease at the time of surgery were excluded. All histological specimens were evaluated by a sarcoma pathologist according to standard recommendations[16]. Tumour size was measured on the histological specimen except in patients who received neoadjuvant treatment, in whom pretreatment CT was used. Mitoses were neither counted in specimens subjected to neoadjuvant treatment, nor in biopsies. Radiological response evaluation was performed using Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1[17]. The study was approved by the local data protection officer and informed consent was obtained from all patients.

Definition of tumour rupture

Tumour rupture was defined as described previously[2]. In brief, the presence of at least one of the following criteria was required: tumour spillage or fracture, piecemeal resection, incisional biopsy, gastric or intestinal perforation at the tumour site, blood-stained ascites at laparotomy or transperitoneal microscopic infiltration of an adjacent organ. Solely among these criteria, incisional biopsy is an intended procedure with no possible relation to tumour biology. For the sake of stringency, the single patient who underwent incisional biopsy was nevertheless retained in the analysis. Minor defects of tumour integrity were not considered rupture, including transabdominal core needle biopsy, peritoneal tumour penetration, superficial peritoneal rupture (iatrogenic) and microscopically involved resection margins. Defects of tumour integrity are illustrated in *Fig. 1*.

Mutation analysis

In the study, mutation analysis was carried out routinely on all intermediate- and high-risk tumours, and selectively on tumours at low or very low risk. In patients who later developed metastatic disease, genotyping of the primary or a recurrent tumour was undertaken. Mutation analysis was performed as described previously[18,19]. Genomic DNA was extracted from formalin-fixed paraffin-embedded or fresh frozen tumour tissue, and analysed by Sanger sequencing. Exons 9, 11, 13 and 17 of the *KIT* gene, and exons 12, 14 and 18 of *PDGFRA* were analysed. Mutations were categorized as deletions, insertions–deletions (indels), substitutions or duplications/insertions according the amino acid changes predicted from the nucleotide sequence. An indel refers to a deletion of amino acids and a concomitant insertion of at least one amino acid, whereas a deletion is an exclusive deletion of at least one amino acid.

Statistical analysis

Relationships between categorical variables were investigated using contingency tables and Pearson's χ^2 or Fisher's exact test. Associations between tumour size or mitotic count (as continuous variables) and tumour genotype were investigated using independent-samples Mann–Whitney *U* test. Multivariable logistic regression analysis was undertaken to ascertain the effects of tumour genotype, tumour size and mitotic count on the likelihood of tumour rupture. Mitoses were not counted after imatinib treatment, and patients who received neoadjuvant imatinib were not included in the multivariable analysis. Data analysis was performed using SPSS version 21.0 (IBM, Armonk, New York, USA). $P < 0{\cdot}050$ was considered statistically significant.

Results

From January 2000 to April 2017, 375 patients underwent surgery for localized gastric or small intestinal GIST, of whom 269 (71·7 per cent) had gastric and 106 (28·3 per cent) had small intestinal tumours. Mutation analysis was not performed in 153 patients, and was not technically successful in 11. Classification of tumour rupture was not possible in two patients. Thus, 209 patients were included in the final study cohort.

From 2000 to 2015, 255 patients with resected, localized gastric GIST and 111 with resected, localized small intestinal GIST were reported to the Cancer Registry of Norway from the catchment area of OUH. For the same interval, the sarcoma database at OUH contains 242 patients with localized gastric and 100 with localized small intestinal GIST who underwent surgery, representing 94·9 and 90·1 per cent respectively of the regional cohort.

Demographic, clinical and histopathological characteristics of the 209 included patients are summarized in *Table 1*.

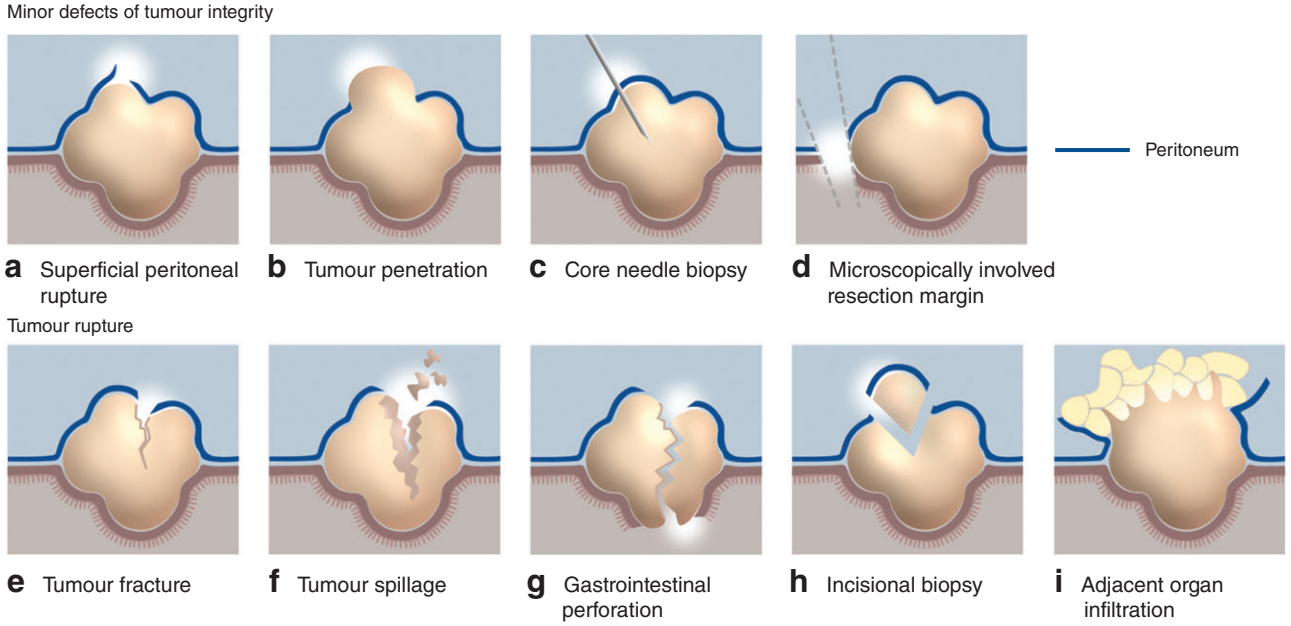

Fig. 1 Illustrations showing **a–d** minor defects of tumour integrity and **e–i** tumour rupture: **a** superficial peritoneal rupture; **b** peritoneal tumour penetration; **c** core needle biopsy; **d** microscopically involved resection margin; **e** tumour fracture; **f** tumour spillage; **g** gastrointestinal perforation; **h** incisional biopsy; and **i** adjacent organ infiltration (microscopic). The definition of tumour rupture also includes piecemeal resection and blood-stained ascites

There were 117 men (56·0 per cent) and median age was 66 (range 14–93) years. One hundred and forty-eight tumours (70·8 per cent) were located in the stomach and 61 (29·2 per cent) in the small intestine. Tumour rupture was recorded in 37 patients (17·7 per cent). Types of tumour rupture are summarized in *Table 2*.

Mutation analysis

KIT mutations were found in 167 tumours (80·0 per cent) and *PDGFRA* mutations in 29 (13·9 per cent); no mutation was discovered in 13 tumours (6·2 per cent) (*Table 1*). All tumours with *PDGFRA* mutations were gastric, and all tumours with *KIT* exon 9 mutations were intestinal. The most frequent types of mutation were deletions and substitutions (each 35·9 per cent), followed by duplications/insertions (12·9 per cent) and indels (9·1 per cent). Deletions and indels were grouped together in the following analyses.

Tumour genotype and rupture

Rupture occurred in 33 of 167 tumours with a *KIT* mutation (19·8 per cent), in two of 29 (7 per cent) with a *PDGFRA* mutation and in two of 13 (15 per cent) with no mutation ($P = 0·256$) (*Table 3*). One patient with a small intestinal GIST and a Lys642Glu *KIT* exon 13 mutation had tumour rupture; otherwise all *KIT* mutations in ruptured tumours involved exon 11. Thirty-two of 37 patients with tumour rupture had *KIT* exon 11 mutations (*Table 3*).

KIT exon 11 mutations and tumour rupture

Among patients with *KIT* exon 11 mutations, 25 of 86 (29 per cent) with a deletion or indel mutation had tumour rupture, compared with five of 50 (10 per cent) for substitutions and two of 19 (11 per cent) for duplications/insertions ($P = 0·014$) (*Table 3*). Tumours with a deletion or indel involving codons 557 and 558 (del557/558) had the highest risk of rupture. Rupture occurred in 17 of 46 tumours (37 per cent) with del557/558 and in 15 of 109 (13·8 per cent) tumours with other exon 11 mutations (relative risk (RR) 2·69, 95 per cent c.i. 1·47 to 4·91; $P = 0·002$). The association was confined to gastric tumours: 12 of 34 gastric GISTs (35 per cent) with del557/558 ruptured compared with six of 77 (8 per cent) with other exon 11 mutations (RR 4·53, 1·85 to 11·06; $P = 0·001$). Rupture was recorded in five of 12 patients with tumours in the small intestine and del557/558 mutation, and in nine of 32 with other exon 11 mutations ($P = 0·475$). The frequency of del557/558 mutations was similar for tumours at both locations: 34 of 111 gastric tumours (30·6 per cent) and 12 of 44

Table 1 Demographic, clinical and histopathological characteristics

	No. of patients*
Age at diagnosis (years)†	66 (14–93)
Sex ratio (F : M)	92 : 117
Tumour location	
Stomach	148 (70·8)
Small intestine	61 (29·2)
Surgery at OUH Sarcoma Centre	159 (76·1)
Emergency surgery	21 (10·0)
Completeness of resection	
R0	175 (83·7)
R1	32 (15·3)
R2	1 (0·5)
Not specified	1 (0·5)
Tumour rupture	
Yes	37 (17·7)
No	172 (82·3)
Tumour size (cm)†	6·2 (0·5–30·0)
Mitotic count (per 50 HPF)†	3 (0–178)
Modified NIH criteria	
Very low	4 (1·9)
Low	52 (24·9)
Intermediate	39 (18·7)
High	113 (54·1)
Unspecified	1 (0·5)
Neoadjuvant treatment	10 (4·8)
Adjuvant treatment	69 (33·0)
Mutation analysis	
KIT exon 9	7 (3·3)
KIT exon 11	155 (74·2)
KIT exon 13	2 (1·0)
KIT exon 17	3 (1·4)
PDGFRA exon 12	2 (1·0)
PDGFRA exon 18	27 (12·9)
No mutation detected	13 (6·2)

*With percentages in parentheses unless indicated otherwise; †values are median (range). OUH, Oslo University Hospital; HPF, high-power fields; NIH, National Institutes of Health.

intestinal tumours (27 per cent) had a del557/558 mutation ($P = 0·846$).

Del557/558 mutations and other risk factors for tumour rupture

Gastric tumours with del557/558 had a median size of 10·9 (range 2·8–30·0) cm *versus* 5·1 (0·5–21·0 cm) for gastric tumours with other genotypes ($P < 0·001$). Gastric tumours with del557/558 also had a higher mitotic count: median 18 (range 2–178) *versus* 3 (0–35) mitoses per 50 high-power fields respectively ($P < 0·001$). Thirty of 34 patients with del557/558 were classified as high risk according to the modified NIH criteria, two were at intermediate risk and one at low risk; it was not possible to classify one patient. All patients with tumour rupture would have satisfied the high-risk criteria based on mitotic count and tumour size only. Thus, including rupture as a risk factor did not influence the classification.

Table 2 Types of tumour rupture in relation to tumour genotype

	Tumour genotype				
	Del557/558 mutation*	Other *KIT* mutation	*PDGFRA* mutation	No mutation detected	Total
Piecemeal resection	4	2	0	0	6
Spillage/fracture	5	3	0	0	8
Incisional biopsy	0	0	0	1	1
Blood-stained ascites	5	4	2	1	12
Perforation	1	3	0	0	4
Adjacent organ infiltration	1	1	0	0	2
Several criteria fulfilled	1	3	0	0	4

*Includes deletions and insertions–deletions involving codons 557 and 558 of the *KIT* gene.

In multivariable logistic regression analysis with del557/558, tumour size and mitotic count as co-variables, del557/558 (odds ratio (OR) 5·29, 95 per cent c.i. 1·06 to 26·31; $P = 0·042$) and tumour size (OR 1·49, 1·24 to 1·80; $P < 0·001$) were associated with an increased likelihood of tumour rupture, whereas mitotic count was not (OR 1·01, 0·98 to 1·04; $P = 0·688$).

Type of tumour rupture and neoadjuvant treatment

The relationship between type of tumour rupture and tumour genotype is outlined in *Table 2*. Fifteen of 37 patients had iatrogenic tumour rupture, rupture occurred spontaneously in 18 patients, and four patients had both iatrogenic and spontaneous rupture. Ten patients received neoadjuvant imatinib, eight with gastric tumours and two with small intestinal tumours.

The median duration of neoadjuvant treatment was 8 (range 1–10) months. Five patients had a partial response (PR) and five had stable disease (SD) as the best radiological response. All patients with SD had tumour shrinkage that did not fulfil the criteria for a PR. Among the six patients with gastric tumours and del557/558 mutation who received neoadjuvant treatment, rupture occurred in one, a 46-year-old man who had an initial PR and whose tumour then progressed after 7 months on imatinib; he underwent surgery with complete tumour excision, but the tumour had ruptured spontaneously and there was also spillage. A *KIT* exon 14 mutation (Thr670Ile) was detected in the surgical specimen. Secondary mutations were not detected in any of the other patients.

Table 3 Mutation analysis and selected clinical and histopathological characteristics

	All patients	Stomach	Small intestine	Tumour size (cm)*	Mitotic count (per 50 HPF)*	Tumour rupture
All patients	209	148 (70·8)	61 (29·2)	6·2 (0·5–30·0)	3 (0–178)	37 (18)
PDGFRA exon 18	27	27 (100)	0 (0)	6·0 (2·5–21·0)	1 (0–19)	2 (7)
Asp842Val	21	21 (100)	0 (0)	6·0 (3·0–21·0)	1 (0–4)	1 (5)
Other	6	6 (100)	0 (0)	n.d.	n.d.	1 (17)
PDGFRA exon 12	2	2 (100)	0 (0)	n.d.	n.d.	0 (0)
KIT exon 9	7	0 (0)	7 (100)	n.d.	n.d.	0 (0)
KIT exon 11	155	111 (71·6)	44 (28·4)	6·5 (0·5–30·0)	5 (0–178)	32 (21)
Del/indel with del557/558	46	34 (74)	12 (26)	9·9 (2·8–30·0)	12 (0–178)	17 (37)
Del/indel not del557/558	40	22 (55)	18 (45)	4·2 (0·5–20·0)	4 (0–106)	8 (20)
Substitutions	50	37 (74)	13 (26)	6·0 (1·3–15·0)	3 (0–24)	5 (10)
Duplications/insertions	19	18 (95)	1 (5)	6·0 (2·0–18·0)	4 (0–16)	2 (11)
KIT exon 13	2	0 (0)	2 (100)	n.d.	n.d.	1 (50)
KIT exon 17	3	3 (100)	0 (0)	n.d.	n.d.	0 (0)
No mutation detected	13	5 (38)	8 (62)	3·8 (1·7–19·0)	1 (0–8)	2 (15)

Values in parentheses are percentages unless indicated otherwise; *values are median (range). HPF, high-power fields; n.d., not determined in groups with fewer than ten patients; del, deletion; indel, insertion–deletion.

Discussion

GISTs with deletions involving codons 557 and 558 of *KIT* exon 11 often have an aggressive phenotype[13–15]. The present population-based study showed that gastric tumours with such deletions also carry a high risk of rupture, an incident that invariably predicts a poor outcome. This increased risk was not restricted to large tumours, suggesting an extended role for mutational analysis in preoperative planning.

Only one previous study has reported the association between tumour genotype and rupture. Using mutation data from the European ConticaGIST database, Wozniak and co-authors[12] detected a statistically non-significant increased risk of rupture in tumours with deletions involving codons 557 or 558 (10 per cent *versus* 6 per cent for other *KIT* exon 11 mutations). However, a consistent definition of rupture was not used, and rupture data were lacking for nearly 20 per cent of the patients. Tumour genotype was also reported in a study of recurrence patterns in 23 ruptured GISTs[1]; seven of 14 patients with *KIT* exon 11 mutations had del557/558. The present data demonstrate an increased risk of rupture only in gastric tumours with del557/558. Among small intestinal tumours, rupture was recorded in five of 12 with del557/558 compared to nine of 32 with other exon 11 mutations ($P = 0{\cdot}475$). In a larger study population, a statistically significant difference might also have been detected for tumours in this location. Gastric GISTs with a *KIT* mutation other than del557/558 had a low risk of rupture (8 of 114, 7·0 per cent), whereas the risk in gastric tumours with del557/558 was similar to the risk in all small intestinal tumours, irrespective of mutations (12 of 34 (35 per cent) *versus* 17 of 61 (28 per cent)). Given the population-based nature of the present cohort, the authors conclude that gastric GISTs with del557/558 have a higher risk of rupture than gastric tumours with other mutations.

According to current guidelines[9,20], preoperative imatinib is indicated if the extent of surgery might be reduced by downsizing or if adequate surgery can be performed more safely after cytoreduction. It is not known whether neoadjuvant imatinib reduces the risk of rupture, as studies on preoperative treatment of locally advanced GIST have not reported on rupture[8,21–24]. It is, however, assumed that neoadjuvant treatment makes tumours more manageable and less prone to rupture when a size reduction is achieved. In the present study, the rupture was iatrogenic, and therefore potentially avoidable, in 15 of 37 patients. Eight patients with gastric tumours were treated before surgery. Although the numbers are small, it is noteworthy that, among these, rupture occurred in only one of six tumours with a del557/558 mutation, and this patient had radiological progression before surgery. A related, and equally unsettled issue, is whether neoadjuvant imatinib attenuates the deleterious consequences of tumour rupture. Hopefully, these are matters that will be explored in future clinical trials, and the present data indicate some questions to be posed.

Tumours with del557/558 normally respond to imatinib treatment[25,26]. In the French BFR14 trial[26] in metastatic GIST, patients with mutations affecting codons 557 and 558 had a higher response rate than patients with other *KIT* exon 11 mutations, with an objective response rate of 81 per cent. However, they developed secondary mutations more rapidly and had significantly reduced progression-free survival. The duration of imatinib response based on tumour genotype has not been studied formally in the neoadjuvant setting, but the overall risk of progression during imatinib

treatment in previous series[8,22,23] has been below 5 per cent. Still, patients with a del557/558 mutation should be followed carefully given the increased risk of tumours with this genotype developing secondary mutations.

Tumours with del557/558 are large, have a high mitotic count and a poor prognosis[13–15]. It could be assumed that these tumours are growing faster and are more necrotic than tumours with other genotypes, resulting in a more fragile structure. Specific downstream signalling events are activated by del557/558[27], indicating that certain uncharacterized phenotypic changes that affect the likelihood of rupture may be induced by distinct *KIT* and *PDGFRA* mutations. In support of this hypothesis, del557/558 remained associated with rupture when size and mitotic count were included in a logistic regression analysis, suggesting an independent risk associated with this mutation. Tumour size is still the most important risk factor, as only one gastric tumour smaller than 10 cm ruptured. While awaiting future clinical studies in locally advanced GIST, the authors suggest that all gastric GISTs larger than 10 cm should be considered for neoadjuvant imatinib, and that preoperative treatment should be standard in the presence of large tumours with a del557/558 mutation. This high-risk feature can be identified in the diagnostic evaluation and should be included in the assessment when neoadjuvant treatment with imatinib is considered.

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