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# Influence of pretreatment with everolimus or sunitinib on the subacute hematotoxicity of $^{177}$Lu-DOTATATE PRRT

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**ABSTRACT**

**Background:** Peptide receptor radionuclide therapy (PRRT) is a validated treatment for somatostatin receptor overexpressing neuroendocrine tumors (NETs). The NETTER-1 trial demonstrated a pronounced positive effect on progression-free-survival compared to high dose somatostatin analogs (SSAs), with a strong tendency toward overall survival benefit. Our aim was to investigate the influence of pretreatment with everolimus and/or sunitinib on subacute hematotoxicity of PRRT. To assess the influence of prior treatment with everolimus/sunitinib might be of clinical relevance due to the link between short-term hematotoxicity and increased incidence of late hematotoxicity.

**Material and methods:** Our single-center retrospective study enrolled all patients treated with $^{177}$Lu-DOTATATE PRRT (1–4 cycles of 7.4 GBq), between November 2013 and July 2018. Patients were assigned to two groups according to their pretreatment: no targeted agents ($N = 41$), or targeted agents (everolimus, sunitinib or both; $N = 41$). The end point was subacute hematotoxicity, defined as the nadir value between the first administration until 3 months after the last administration, using the CTCAE 4.03 classification. The impact of splenectomy was also explored.

**Results:** Eighty percent of patients had a primary gastroenteropancreatic NET. No statistically significant differences in severe subacute hematotoxicity were seen in the pretreated group vs. the naive group for hemoglobin (grade 3/4: 12% vs. 22%), neither for leucocytes (grade 3/4: 10% vs. 7%), neutrophils (grade 3/4: 5% vs. 7%), lymphocytes (grade 3/4: 49% vs. 37%) and platelets (grade 3/4: 15% vs. 15%). Furthermore, we observed significantly lower toxicity for total white blood cells, lymphocytes and platelets in the subgroup that had splenectomy ($N = 12$). Limitations of this study include the potential bias in lack of use of targeted agents in patients more susceptible to toxicity, and the limited number of patients and events.

**Conclusions:** In a patient cohort with NET pretreated with everolimus and/or sunitinib, we could not demonstrate a significant effect of prior/pretreatment with everolimus and/or sunitinib on the subacute hematotoxicity of $^{177}$Lu-DOTATATE PRRT.

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## Introduction

Neuroendocrine tumors (NETs) are a heterogeneous group of relatively rare neoplasms arising from frequently hormonally secreting/active neuroendocrine cells throughout the body [1]. Metastatic disease is very prevalent in gastroenteropancreatic NETs (GEP-NETs) [2,3]. At initial diagnosis, 40–50% of the patients present with distant metastases, with increasing prevalence over time depending on initial disease stage. Treatment is often challenging, given the heterogeneity of NETs, the individual disease complexity and the variety of treatment options. Discussion of these cases in a multidisciplinary tumor board is mandatory, after accurate imaging and pathology review [4]. Treatment with somatostatin analogs (SSAs) is a cornerstone for control of hormonal secretion and symptoms, and for its antiproliferative effect in somatostatin receptor (SSTR) positive patients.

In case of progression, therapy is often switched to targeted agents. The mammalian target of rapamycin (mTOR) inhibitor everolimus is approved for treatment of unresectable or metastatic well or moderately differentiated primary pancreatic NET and well differentiated (grade 1 or 2) unresectable or metastatic nonfunctional gastrointestinal or pulmonary NET. The Radiant-3 and -4 trial showed a significant improvement in progression-free survival in these patient groups [5,6]. Therapy-related adverse effects include bone marrow depression (all grades: ~16% anemia, ~13% thrombocytopenia; no neutropenia), mild to moderate stomatitis, rash, diarrhea, fatigue, hyperglycemia and infections,

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with 5-7% of patients experiencing grade 3/4 toxicity [7,8]. The multiple tyrosine kinase inhibitor sunitinib is approved for unresectable or metastatic, well-differentiated pancreatic NETs. It improved progression-free survival, overall survival, and the objective response rate among patients in a multinational randomized placebo-controlled phase 3 trial [9]. Its major side effects are bone marrow depression (all grades: ~29% neutropenia, ~17% thrombocytopenia; no anemia), fatigue/asthenia, hand-foot syndrome, hypertension, hypothyroidism and diarrhea, with ~5% of patients experiencing grade 3/4 toxicity, neutropenia being the most frequent one [7,10].

If patients are progressive during first-line treatment with SSAs, peptide receptor radionuclide therapy (PRRT) is another validated treatment for SSTR-overexpressing NETs [11]. The phase III NETTER-1 trial has demonstrated a pronounced positive effect on progression-free-survival compared to high dose SSAs in metastatic midgut NETs, with a strong tendency toward overall survival benefit [12].

In general, PRRT is very well tolerated. Acute adverse effects (occurring within 24 h) after the administration of the radiopharmaceutical are nausea (in 25%) and vomiting (10%). These are caused by the infusion of amino acids, which are administered to prevent renal toxicity. These acute gastrointestinal symptoms can be well treated with anti-emetic drugs. Subacute hematological toxicity (WHO grade 3 or 4) due to irradiation of blood cells and hematopoietic tissue develops in about 5–10% of patients. The nadir normally occurs 4–6 weeks after each treatment, generally followed by a recovery phase [11,13,14].

Known risk factors significantly associated with hematological toxicity are: poor renal function, white blood cell (WBC) count $<4.0 \times 10^9$/L, age over 70 years, extensive tumor mass and high SSTR-expression by the tumor [14].

The groups from Bonn and Rotterdam reported long-term hematotoxicity rates of 1% and 4%, respectively [15,16], consisting mainly of persistent hematological dysfunction (PHD), myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). In a small French cohort, heavily pretreated with alkylating chemotherapy, four out of 20 patients (20%) developed MDS or AML [17]. Recent results from the group of Melbourne emphasize the possibility of preexisting biological or genetic susceptibility to radiation as a contributing factor both for development of therapy-related myeloid neoplasms [18]. Subacute hematotoxicity has been linked to increased incidence of late hematotoxicity, one of the major long-term toxicities of PRRT [15,16]. This pronounced effect raised awareness about pretreatment, because it may compromise the safety and future applicability of PRRT. Especially in patients experiencing early hematological toxicity after PRRT, regular and prolonged monitoring of blood counts is mandatory. Fewer data are available on long-term renal function. Surveillance showed only a slight reduction of glomerular filtration rate (GFR) over time (~2 mL/min/m$^2$ per year) [19].

We hypothesized that previous treatment with agents could worsen the subacute hematotoxicity of PRRT, since both targeted agents can cause bone marrow depression. The aim of this work is to determine the influence of pretreatment with everolimus and/or sunitinib on subacute hematotoxicity of $^{177}$Lu-DOTATATE PRRT. In addition, the protective impact of splenectomy on the development of acute hematotoxicity was assessed.

## Material and methods

### Patients

This study is a single-center retrospective study based on patient records. Patients were treated in a routine clinical setting at the Nuclear Medicine unit of the University Hospital in Leuven, Belgium between November 2013 and July 2018. Patients with histologically proven SSTR overexpressing tumors were eligible for PRRT if following criteria were met: (1) sufficient SSTR-expression on $^{68}$Ga-DOTATATE-PET/CT, i.e., tracer accumulation in the tumoral lesions at least as high as in the normal liver parenchyma (grade 3 or 4 according to the Krenning scale). The Krenning scale ranges from grade 0 (no uptake by tumor) to grade 4 (very intense uptake by tumor), with higher grades indicating a higher level of expression of SSTRs. The highest grade per patient was reported [12]; (2) disease progression under treatment with SSAs and/or targeted agents; (3) the Karnofsky performance status score of at least 50; (4) renal clearance of more than 50 mL/min/1.73 m$^2$; (5) hemoglobin level of more than 8.0 g/dL, total WBC count of more than 2000 per mm$^3$, platelet count of more than 100,000 per mm$^3$, total bilirubin level of less than three times the upper limit of the normal range, serum albumin level of more than 30 g/L. Other previous treatment options (chemotherapy, liver directed transarterial therapy – bland embolization, chemoembolization or radioembolization – or previous PRRT) were included. Contraindications for PRRT were possible surgery with curative intent, uncontrolled congestive heart failure, concomitant use of nephrotoxic medication, previous radiation therapy to more than 25% of the red bone marrow, and pregnancy.

The Ethics Committee Research UZ/KU Leuven approved the use of patient records for this retrospective study.

### PRRT and previous treatment with targeted agents

The intended cumulative activity was 29.6 GBq, administered in four cycles of 7.4 GBq with an interval of 8 weeks. Reasons to reduce the injected activity or end the therapy were persistent or recurrent grade 3 or 4 hematotoxicity and disease progression. To prevent renal toxicity, an intravenous infusion of 2 L of an amino acid solution (Proteinsteril Hepa 8% (Fresenius, Bad Homburg, Germany), with among others 6.88 g/L lysine and 10.72 g/L arginine) was started at least 30 min before the administration of $^{177}$Lu-DOTATATE, and continued up to 4 h later. To prevent amino-acid induced nausea, ondansetron 8 mg was injected intravenously before the $^{177}$Lu-DOTATATE and an continuous infusion of alizapride was given. The radiopharmaceutical was administered in 15 min using a second pump system.

According to their pretreatment with targeted agents, patients were assigned to two groups: a group which was pretreated with everolimus, sunitinib or both ('E/S pretreated' group), and a group which was not treated with targeted agents ('E/S naive' group). Treatment with everolimus and sunitinib was sequential and never simultaneous.

### Toxicity assessment

The primary end point was the effect of pretreatment with targeted agents on the subacute hematotoxicity, defined as the nadir value during the window from the administration of the first PRRT cycle until 3 months after the last cycle, using the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 classification. Blood samples were taken the morning of each treatment and 4 weeks after each administration, at the oncological follow-up consultation, or more frequently, if clinically indicated. Other side effects, such as acute nausea and vomiting, subacute fatigue and long-term hematological or renal toxicity were not assessed.

The association of a number of clinical factors with subacute hematotoxicity was also assessed. These factors included age, total WBC and impaired kidney function [14,20], as well as pretreatment with the targeted agents everolimus and/or sunitinib, the duration of either of these pretreatments and the interval between the pretreatment and start of PRRT. We also looked into the possible effect of the number of cycles of PRRT that were administered and the tumor burden in the liver and bone/bone marrow. The possible protective impact of splenectomy on the development of acute hematotoxicity was also assessed.

### Statistical analysis

Both absolute change to the nadir and the relative change to the nadir were computed.

The difference between groups with regards to continuous or ordinal outcome variables was tested by the Mann–Whitney *U* test. The association between two continuous or ordinal variables was estimated by the Spearman correlation coefficient.

No correction for multiple testing was performed given the exploratory nature of the study. All tests are two-sided; a 5% significance level was adopted for all tests.

Analyses have been performed using SAS software (version 9.4 of the SAS System for Windows) SAS Institute Inc., Cary, NC, USA).

## Results

### Patient characteristics

Ninety consecutive patients were treated with $^{177}$Lu-DOTATATE PRRT. The records of all patients were evaluated. In eight patients, data were lacking (see CONSORT diagram in Supplemental Figure 1), hence they were excluded from the study. The patient characteristics are summarized in Table 1. All NETs showed high $^{68}$Ga-DOTATATE-uptake on PET/CT (grade 3 or 4 according to the Krenning scale). The majority of the patients had primary GEP-NETs (80%), and most tumors were WHO grade 1 (39%) or grade 2 (56%) based on the Ki-67 index. Mean age at the start of PRRT was 64 years (range 24–84). The tumor burden in liver, bone, lymph nodes and other organs is shown in Supplemental Table 1.

Table 1. Baseline patient demographics.

| Characteristic | All patients ($N = 82$) | E/S naive group ($N = 41$) | E/S pretreated group ($N = 41$) |
|---|---|---|---|
| Sex, no. (%) | | | |
| Male | 42 (51%) | 18 (44%) | 24 (59%) |
| Female | 40 (49%) | 23 (56%) | 17 (41%) |
| Age, years (mean ± SD) | 64 ± 12 | 61 ± 11 | 62 ± 13 |
| ≤70 years old, no. (%) | 67 (82%) | 35 (85%) | 32 (78%) |
| >70 years old, no. (%) | 15 (18%) | 6 (15%) | 9 (22%) |
| Primary tumor site, no. (%) | | | |
| Small intestine | 37 (45%) | 27 (66%) | 10 (24%) |
| Pancreas | 25 (30%) | 2 (5%) | 23 (56%) |
| Unknown primary | 7 (9%) | 3 (7%) | 4 (10%) |
| Lung | 5 (6%) | 3 (7%) | 2 (5%) |
| Colon | 4 (5%) | 3 (7%) | 1 (2%) |
| Paraganglioma | 2 (2%) | 1 (2%) | 1 (2%) |
| Meningioma | 1 (1%) | 1 (2%) | 0 (0%) |
| Pheochromocytoma | 1 (1%) | 1 (2%) | 0 (0%) |
| Tumor burden[a] | | | |
| Limited tumor mass, no. (%) | 49 (60%) | 26 (63%) | 23 (56%) |
| Extensive tumor mass, no. (%) | 33 (40%) | 15 (37%) | 18 (44%) |
| Krenning grade, no. (%) | | | |
| 3 | 8 (10%) | 4 (10%) | 4 (10%) |
| 3–4 | 26 (32%) | 9 (22%) | 17 (42%) |
| 4 | 48 ( 59%) | 28 (69%) | 20 (49%) |
| Renal function (GFR from $^{51}$Cr-EDTA) clearance | | | |
| ≤60 mL/min, no. (%) | 13 (16%) | 6 (15%) | 7 (17%) |
| >60 mL/min, no. (%) | 69 (84%) | 35 (85%) | 34 (83%) |
| Leukocytosis | | | |
| $<4.0 \times 10^9$/L, no. (%) | 6 (7%) | 1 (2%) | 5 (12%) |
| $\geq 4.0 \times 10^9$/L, no. (%) | 76 (93%) | 40 (98%) | 36 (88%) |
| Splenectomy, no. (%) | | | |
| No | 70 (85%) | 39 (95%) | 31 (76%) |
| Yes | 12 (15%) | 2 (5%) | 10 (24%) |
| Ki-67[b] | ($N = 70$) | ($N = 31$) | ($N = 39$) |
| Median | 4.0 | 2.0 | 6.0 |
| IQR | 2.0–10.0 | 1.0–5.0 | 3.0–12.0 |
| Range | 1.0–30.0 | 1.0–25.0 | 1.0–30.0 |
| WHO grade[b], no. (%) | | | |
| Grade 1 | 27 (39%) | 18 (58%) | 9 (23%) |
| Grade 2 | 39 (56%) | 11 (35%) | 28 (72%) |
| Grade 3 | 4 (6%) | 2 (6%) | 2 (5%) |
| Treatment related parameter | | | |
| Number of cycles of PRRT, no. (%) | | | |
| 4 | 69 (84%) | 35 (85%) | 3 (83%) |
| 3 | 5 (6%) | 2 (5%) | 3 (7%) |
| 2 | 7 (9%) | 4 (10%) | 3 (7%) |
| 1 | 1 (1%) | 0 (0%) | 1 (2%) |

[a]Extensive tumor mass was defined as patients having >5 lesions in at least two of the following organs (liver, bone, adenopathies or other) and at least one additional lesion in another organ.
[b]Ki-67 was not available for all patients. Three categories are defined: grade 1 (Ki-67 ≤ 2%), grade 2 (Ki-67 > 2–20%) and grade 3 (Ki-67 > 20%) NET.

### Pretreatment and treatment

Patients with primary pancreatic, lung and nonfunctional intestinal NETs were eligible for everolimus. Sunitinib was only indicated for primary pancreatic NETs. Forty-one patients (50%) were pretreated with one or both of these

targeted agents, with a relatively similar duration for both (see Supplemental Table 2 and Supplemental Figure 2). The characteristics of the E/S pretreated and the E/S naive groups were comparable, except for a higher incidence of grade 2 tumors in the pretreated group (28 vs. 11) and a history of splenectomy in the pretreated group (10 vs. 2). Regarding PRRT, the vast majority of patients (84%) received the intended cumulative activity of 29.6 GBq, administered in four cycles.

### Maximal observed acute hematotoxicity according to CTCAE 4.03

An overview of the maximal toxicity grades is shown in Table 2. For clinical relevance, mild hematological toxicities (grades 1 and 2) were pooled and the more severe toxicities (grade 3 and 4) were reported separately.

Grade 3/4 hematotoxicity (red blood cells, total WBCs, platelets) was observed in seven of the 41 E/S pretreated patients (17%) vs. 11 of the 41 E/S naive patients (27%; $p = .42$). When taking the differential WBC count into account, 19 patients in the E/S pretreated group (46%) and 18 in the E/S naive group (44%; $p = 1.0$) showed some form of grade 3/4 hematotoxicity.

From the start of PRRT until 3 months after the last treatment, all patients showed some degree of anemia. Twelve patients (15%) had a grade 3 anemia and only two patients (2.4%) developed a grade 4 anemia. More than a third of the patients (39%) did not show any WBC toxicity. In 43 of the patients (52%), a mild leucopenia and in seven (8.5%) patients a grade 3 leucopenia was observed. Regarding the neutrophils, the maximal observed toxicity was grade 3 in seven patients (8.5%). Lymphocytopenia was more pronounced, with 42 patients (51%) showing a grade 1–2 toxicity, 30 patients (37%) showing a grade 3 toxicity, and five patients (6.1%) a grade 4 toxicity. Almost half of the patients (46%) developed a mild thrombocytopenia (G1/2). Grade 3 and 4 toxicity were observed in six patients (7%) each. No significant differences were seen between the group pretreated with everolimus and/or sunitinib vs. the naive group.

**Table 2.** Maximal toxicity after start PRRT until 3 months after last administration.

| Toxicity grade, no. (%) | All patients ($N = 82$) | E/S naive group ($N = 41$) | E/S pretreated group ($N = 41$) |
|---|---|---|---|
| Hemoglobin | | | |
| 0 | 0 (0%) | 0 (0%) | 0 (0%) |
| 1–2 | 68 (83%) | 36 (88%) | 32 (78%) |
| 3 | 12 (15%) | 4 (10%) | 8 (20%) |
| 4 | 2 (3%) | 1 (2%) | 1 (2%) |
| 1–4 | 82 (100%) | 41 (100%) | 41 (100%) |
| Leucocytes | | | |
| 0 | 32 (39%) | 17 (41%) | 15 (37%) |
| 1–2 | 43 (52%) | 20 (49%) | 23 (56%) |
| 3 | 7 (9%) | 4 (10%) | 3 (7%) |
| 4 | 0 (0%) | 0 (0%) | 0 (0%) |
| 1–4 | 50 (61%) | 24 (59%) | 26 (63%) |
| Neutrophils | | | |
| 0 | 35 (43%) | 18 (44%) | 17 (46%) |
| 1–2 | 42 (51%) | 20 (49%) | 21 (51%) |
| 3 | 5 (6%) | 4 (10%) | 3 (7%) |
| 4 | 0 (0%) | 0 (0%) | 0 (0%) |
| 1–4 | 48 (59%) | 24 (59%) | 24 (59%) |
| Lymphocytes | | | |
| 0 | 5 (6%) | 1 (2%) | 4 (10%) |
| 1–2 | 42 (51%) | 20 (49%) | 22 (54%) |
| 3 | 30 (37%) | 17 (41%) | 13 (32%) |
| 4 | 5 (6%) | 3 (7%) | 2 (5%) |
| 1–4 | 77 (94%) | 40 (98%) | 37 (90%) |
| Platelets | | | |
| 0 | 32 (39%) | 16 (39%) | 16 (39%) |
| 1–2 | 38 (46%) | 19 (46%) | 19 (46%) |
| 3 | 6 (7%) | 2 (5%) | 4 (10%) |
| 4 | 6 (7%) | 4 (10%) | 2 (5%) |
| 1–4 | 50 (15%) | 25 (61%) | 25 (61%) |

Mild toxicities (grades 1 and 2) are combined in one category. Grades 3 and 4 toxicity are reported separately for clinical relevance.

### Influence of targeted agent pretreatment on the evolution of the complete blood count

Given the setting of advanced disease with a history of multiple previous treatments, it is not surprising that some patients can develop chronic hematotoxicity. One out of our 82 patients (1%) developed AML. To isolate the subacute toxic effect of PRRT, we looked into the evolution of the blood counts between the start of PRRT and the nadir value during treatment and the 3 month follow-up period (Table 3).

The decrease in hemoglobin due to the PRRT was different between both subgroups (median absolute difference –1.9 g/dL in the naive group vs. –1.1 g/dL in the pretreated group ($p$=.016)). The relative difference was also significantly more pronounced in the naive group: –13% vs. –10% ($p$=.024). For the leucocytes, the median difference was similar for both groups: $-3.1 \times 10^9$/L vs. $-2.3 \times 10^9$/L, for the E/S naive and the E/S pretreated group, respectively ($p$=.14). The median absolute decrease of the neutrophils was significantly more pronounced in the group that was not pretreated: $-2.4 \times 10^9$/L vs. $-1.4 \times 10^9$ ($p$=.03). For the lymphocytes, there was a significantly more pronounced effect in the E/S naive group in terms of absolute decrease (median –0.9 vs. $-0.6 \times 10^9$/L; $p$=.018), relative decrease (median –65% vs. –47%; $p$=.001) and difference in grade (median 2.0 vs. 1.0; $p$<.001), contrary to our hypothesis.

**Table 3.** Differences (in absolute values, relative values and grade) in hematological levels between baseline and the nadir value observed during the window from first administration of PRRT until 3 months after last PRRT administration.

| Blood cell type, median (IQR) | E/S naive group ($N = 41$) | E/S pretreated group ($N = 41$) | $p$ Value |
|---|---|---|---|
| Hemoglobin | | | |
| Absolute | –1.9 (–3.1; –1.0) | –1.1 (–1.8; –0.4) | **.016** |
| Relative (%) | –13.4 (–21.3; –7.9) | –10.1 (–13.9; –3.1) | **.024** |
| Leucocytes | | | |
| Absolute | –3.1 (–4.1; –2.2) | –2.3 (4.2; –1.8) | .143 |
| Relative (%) | –48.1 (–56.4; –32.6) | –44.0 (–52.5; –30.2) | .326 |
| Neutrophils | | | |
| Absolute | –2.4 (–3.1; –1.7) | –1.4 (–3.0; –0.8) | **.030** |
| Relative (%) | –50.0 (–55.9; –39.2) | –39.5 (–60.0; –22.9) | .121 |
| Lymphocytes | | | |
| Absolute | –0.9 (–1.3; –0.7) | –0.6 (–1.1; –0.3) | **.018** |
| Relative (%) | –65.1 (–76.7; –55.1) | –47.2 (–64.4; –37.3) | **.001** |
| Platelets | | | |
| Absolute | –97 (–204.0; –44.0) | –135 (–207.0; –77.0) | .197 |
| Relative (%) | –41.2 (–68.7; –23.0) | –53.3 (–66.7; –36.5) | .495 |

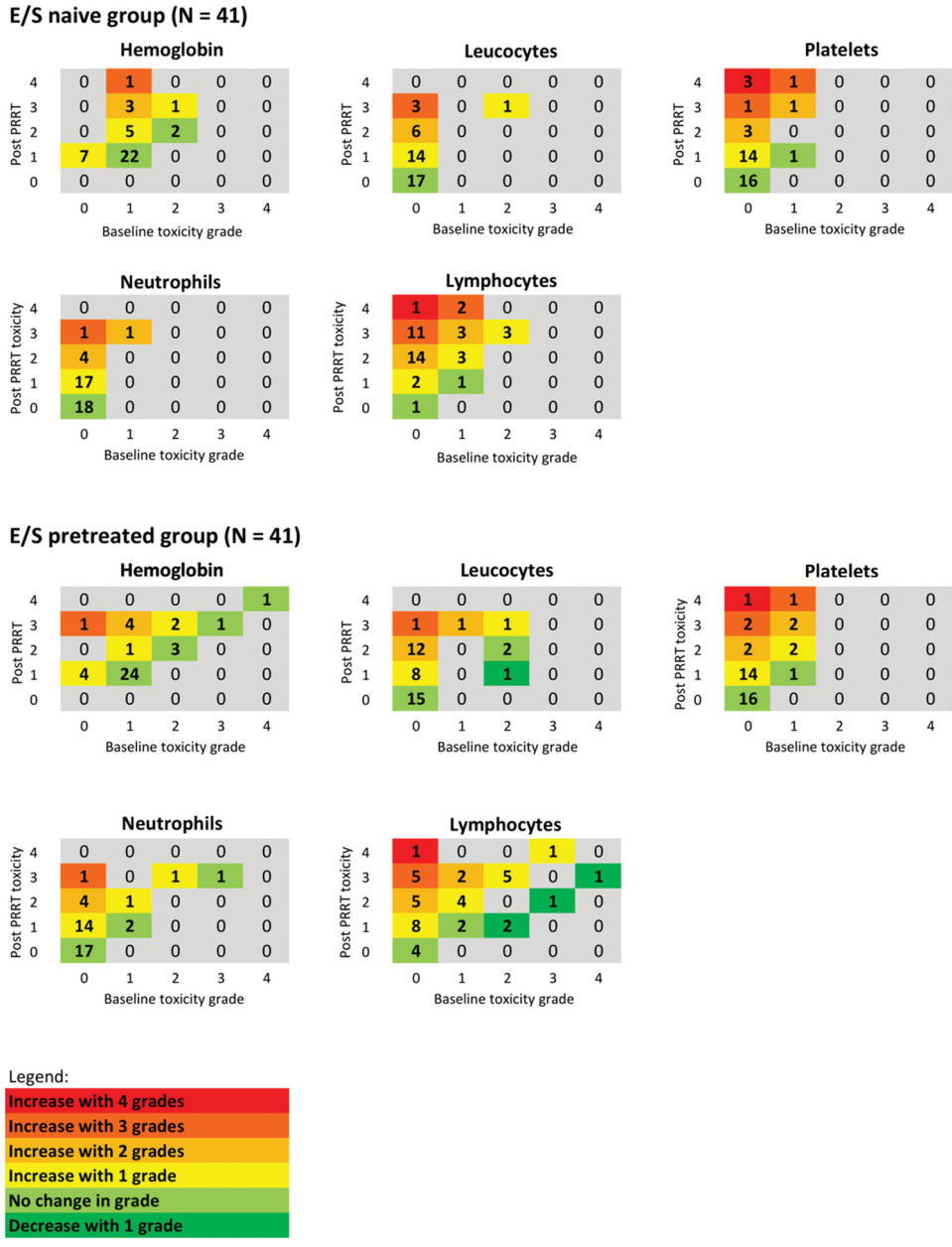


**Figure 1.** The evolution of the toxicity grade per patient with regard to the pretreatment.

There was no significant difference in decrease of the platelet levels.

The evolution of the toxicity grade from baseline to nadir, according to E/S pretreatment status, is shown in Figure 1. In general, there is no increase in specific toxicity change in the E/S pretreated group compared to the E/S naive group.

### *Effect of clinical parameters on occurrence of subacute hematotoxicity*

Table 4 shows the association between a number of clinical factors and subacute hematotoxicity. Increasing tumor burden in the liver and bone is significantly associated with platelet toxicity. The duration of pretreatment with sunitinib was significantly associated with an increase in lymphocytes; an effect in opposition with our hypothesis. A decreased renal function (GFR $<$ 60 mL/min) was associated with a relative decrease of the platelets and the grade of platelet toxicity. We also observed a significant association between a lower WBC count at baseline (in six patients), and lower WBC toxicity. This might be due to the fact that a hematotoxic pretreatment (such as chemotherapy or targeted therapy) was stopped. During PRRT, the patients' bone marrow could partially recover.

For the other defined covariates (age, overall tumor burden (limited vs. extensive), number of cycles of PRRT, duration of the pretreatment with everolimus, interval between the end of the pretreatment and start of PRRT), no significant associations were found.

In patients with a history of splenectomy, we observed significantly less toxicity on total WBCs (median grade 0 vs. 1.0 in the splenectomy group vs. no splenectomy; $p$=.009),

Table 4. Association of risk factors with toxicity.

| Risk factor | $\rho$ | 95% confidence interval | $p$ Value |
|---|---|---|---|
| Tumor burden, liver ($N = 82$) | | | |
| Platelets, absolute | −0.228 | (−0.42; −0.01) | **.039** |
| Platelets, relative | −0.205 | (−0.40; 0.01) | .065 |
| Platelets, grade | 0.078 | (−0.14; 0.29) | .485 |
| Tumor burden, bone ($N = 82$) | | | |
| Platelets, absolute | −0.058 | (−0.27; 0.16) | .608 |
| Platelets, relative | −0.195 | (−0.40; 0.02) | N.S. |
| Platelets, grade | 0.265 | (0.05; 0.45) | **.016** |
| Duration of sunitinib treatment ($N = 25$) | | | |
| Lymphocytes, absolute | 0.448 | (0.06; 0.71) | **.024** |
| Lymphocytes, relative | 0.197 | (−0.22; 0.55) | .349 |
| Lymphocytes, grade | −0.032 | (−0.42; 0.37) | .882 |
| Interval between pretreatment with sunitinib and start PRRT ($N = 25$) | | | N.S. |
| All parameters | [−0.264; 0.012] | / | All $> .05$ |
| Duration of everolimus treatment ($N = 37$) | | | N.S. |
| All parameters | [−0.397; 0.334] | / | All $> .05$ |
| Interval between pretreatment with everolimus and start PRRT ($N = 37$) | | | N.S. |
| All parameters | [−0.315; 0.062] | / | All $> .05$ |
| Number of cycles of PRRT ($N = 82$) | | | |
| Lymphocytes, absolute | −0.233 | (−0.427; −0.016) | **.035** |
| All other parameters | [−0.196; 0.068] | / | All $> .05$ |

All these analyses are univariate. The association between the continuous and ordinal variables is estimated by the Spearman correlation coefficient ($\rho$). A negative correlation means a decrease for a higher exposure to a risk factor. A positive correlation means an increase for a higher exposure to a risk factor. The Spearman correlation coefficient ($\rho$), 95% confidence interval and $p$ value were only reported for statistically significant associations between risk factors with toxicity.

lymphocytes (median grade 0.5 vs. 2.0; $p$=.007) and platelets (median grade 0 vs. 1.0; $p$=.001). Similar results were observed in an analysis of patients without splenectomy ($N = 70$; data not shown).

## Discussion

The first-line systemic treatment in the majority of patients with advanced NET is therapy with SSAs. This is an established therapy for antiproliferative purposes in lower grade GEP-NETs (Ki-67 up to 10%), based on two placebo-controlled trials (the PROMID study and the CLARINET study) [20,21]. SSAs may also be used in NETs of other sites (e.g., bronchial NET), if SSTR-overexpression is present and if the tumor grows slowly [3].

Due to reimbursement regulations within the Belgian healthcare system, treatment with targeted agents is mandatory before $^{177}$Lu-DOTATATE PRRT is reimbursed. This is in contrast to many other published PRRT cohorts.

Recently, data have been published [22–25] regarding the timing of targeted radionuclide therapy in combined-modality regimens (also called sequencing). These multimodal treatment combinations are designed to improve outcome, overcome the development of resistance and to minimize off-target effects.

To the best of our knowledge, this is the first study to assess the influence of pretreatment with everolimus and/or sunitinib on subacute hematotoxicity of PRRT (between first PRRT treatment and till 3-months follow-up).

In our study cohort, 18% developed a subacute grade 3/4 anemia. Nine percent developed a grade 3 leukopenia and 6% developed a grade 3 neutropenia. No grade 4 leukopenia or neutropenia was observed. In 43% of the patients, we observed a grade 3/4 lymphocytopenia and 14% of the patients developed a grade 3/4 thrombocytopenia. Our overall toxicity levels are comparable to those reported in other studies [13,14]. At baseline, the majority of the patients showed mild anemia (hemoglobin decreased but $>$10.0 g/dL). Six out of the 82 patients (7%) showed mild leukopenia (grade 1–2). Five patients (6%) showed a mild neutropenia, while one other patient a grade 3 neutropenia. Mild lymphocytopenia was present in 27 patients (33%) at baseline, while severe lymphocytopenia (grade 3–4) was present in three patients (4%). Ten patients showed a grade 1 thrombocytopenia (12%).

Only two patients (3%) needed blood transfusion (grade 3) after PRRT, while none needed granulocyte-colony stimulating factors or platelets transfusion.

Maximal subacute leucopenia and thrombocytopenia were not significantly different in both groups. Remarkably, the E/S naive group showed a more pronounced decrease in the other hematological parameters, contrary to our hypothesis. For the hemoglobin, we observed slightly lower baseline values in the E/S pretreated group. The difference between the baseline level and the nadir value was significantly smaller in the pretreated patients, as compared to the other group. We also observed a paradoxically less pronounced decrease in the lymphocytes in the pretreated group, with similar baseline values. The pretreatment with targeted agents did not have a relevant influence on the neutrophil toxicity. If we look at patient-level grade 3/4 toxicity, 18% of our patients experience acute grade 3/4 hematotoxicity: 11% in the E/S naive group and 7% in the E/S pretreated group. Further research in other treated patient cohorts is warranted to confirm these findings in other cohorts and to unravel its mechanism. A large multicenter study in grade 3 neuroendocrine neoplasms ($n = 149$) [25] reported an incidence of 6% of (sub)acute grade 3/4 hematotoxicity (regarding hemoglobin, total WBC count and platelets; no values reported for differential WBC count) after PRRT. These values are in line with our findings. Carlsen et al. reported similar incidence of toxicity for patients receiving PRRT as first line vs. later line of treatment (after chemotherapy or targeted therapy, including everolimus and sunitinib) [26].

Similar to the findings of the Rotterdam group [14], we observed a modestly, but significant, more pronounced platelet drop in patients with a higher tumor burden in the liver and bone. A possible explanation could be that a larger blood volume is irradiated in hypervascular metastases and the liver is one of the organs with the highest tumor bulk in many patients.

Other clinical factors, such as the number of cycles of PRRT given, the duration of the pretreatment with everolimus and the interval between the end of the pretreatment and start of PRRT were not significantly associated with subacute hematotoxicity.

We also looked into the possible protective effect of splenectomy to develop hematological toxicity in patients receiving $^{177}$Lu-DOTATATE. This seems plausible from a physiologic perspective since the spleen is a major reservoir of blood cells and has high uptake of the radiopharmaceutical. Blood cells passing through the spleen could be irradiated and damaged leading to a reduction in peripheral blood cell counts. In our study, only one of the 12 patients with a history of splenectomy developed clinically relevant subacute hematotoxicity. Furthermore, we observed a significantly lower toxicity in these patients compared to those who did not have a splenectomy for total WBCs, lymphocytes and platelets. As this is a retrospective study and there are potential confounders, this is not demonstrative of a protective effect. However, our results are in line with the data from two other studies [14,15] reporting a possible protective effect of splenectomy.

### *Link to increased late hematotoxicity*

The groups from Bonn and Rotterdam reported long-term hematotoxicity rates of 1% and 4%, respectively [15,16], consisting mainly of PHD, MDS and AML. Bergsma et al. observed that patients treated with PRRT had 2.7 times higher relative risk of developing PHD than patients not treated with PRRT, with a median latency period of 41 months. Also, a trend was observed in which subacute hematologic toxicity (grade 3–4) during PRRT was more frequent in patients with PHD than in those without ($p$=.053). In a small French cohort, heavily pretreated with alkylating chemotherapy, four out of the 20 patients (20%) developed MDS or AML, diagnosed 30, 31, 54 and 70 months, respectively, after PRRT [17]. The patients who developed MDS or AML had received more chemotherapy and more alkylating agents than the other patients ($p$=.001). Three of the four patients (75%) with MDS/AML developed early hematological toxicity (grade 3–4 thrombocytopenia) during PRRT, vs. two out of the 16 (13%) patients without late term hematotoxicity ($p$=.03). This pronounced effect raised awareness about pretreatment, because it may compromise the safety and future applicability of PRRT. The stochastic effects after PRRT remain unexplained for a large extent. Recent results from the group of Melbourne [18] raise the possibility of preexisting biological or genetic susceptibility to radiation as a contributing factor for development of therapy-related myeloid neoplasms. The most frequent karyotypic and genetic abnormalities in their patient population of PRRT-related myeloid neoplasms were abnormalities involving chromosome 5 and/or 7 and mutations in the TP53 gene. Especially in patients experiencing subacute hematological toxicity after PRRT, regular and prolonged monitoring of blood counts is mandatory.

### *Toxicity and contraindications of everolimus and sunitinib*

In patients with severe preexistent pulmonary disease, uncontrolled diabetes or systemic infections such as tuberculosis everolimus is contra-indicated [8]. In patients with known dental or skin problems, or metabolic syndrome, preventive measures should be taken before initiating the therapy. Since everolimus is a substrate of CYP3A4, caution is also warranted when prescribing other drugs, such as systemic corticosteroids for treatment of everolimus-associated adverse events [7].

Preexistent diarrhea, as a symptom of the NET, can be aggravated by the introduction of sunitinib. This might form a contra-indication [10]. To prevent hand-foot syndrome, preexistent calluses or hyperkeratotic areas should be treated. Screening for hypertension and thyroid dysfunction is mandatory before starting sunitinib [7].

### *Limitations*

Limitations of this study are its retrospective nature, potential bias in patient selection for using targeted agents, e.g., in patients more susceptible to toxic effects or in which their use is contra-indicated, and the limited number of patients and events.

## Conclusions

In a patient cohort pretreated with everolimus and/or sunitinib, we could not demonstrate a significant negative influence of pretreatment on the subacute hematotoxicity of $^{177}$Lu-DOTATATE PRRT. With regard to the optimal sequence of approved treatments in NET after failure of first line SSAs, this study does not provide arguments to place everolimus and/or sunitinib treatment after PRRT.

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## Disclosure statement

Christophe M. Deroose has been a consultant for Novartis, Terumo, AAA, Ipsen, Sirtex and Bayer outside the scope of the submitted work. Karolien Goffin has been a consultant for Bayer, Lightpoint Medical and Blue Earth Pharmaceuticals outside the scope of the submitted work.

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