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CASE REPORT



## NGS-analysis to the rescue: dual checkpoint inhibition in metastatic osteosarcoma – a case report and review of the literature

Laure Nuytemans <sup>a</sup>, Gwen Sys <sup>b,c</sup>, David Creyten <sup>c,d</sup> and Lore Lapeire <sup>a,c</sup>

<sup>a</sup>Department of Medical Oncology, Ghent University and Ghent University Hospital, Ghent, Belgium; <sup>b</sup>Department of Traumatology and Orthopedics, Ghent University and Ghent University Hospital, Ghent, Belgium; <sup>c</sup>Cancer Research Institute Ghent (CRIG), Ghent University, Ghent, Belgium; <sup>d</sup>Department of Pathology, Ghent University and Ghent University Hospital, Ghent, Belgium

### ABSTRACT

**Background:** The prognosis and treatment of metastatic osteosarcoma have not changed in the last decades and the responses to chemotherapy in this setting are disappointing. In the past years, immunotherapy has found its place in the treatment of different tumor types. Its role in the treatment of sarcomas, and in particular osteosarcoma, is less clear. Next-generation sequencing (NGS) can help identify patients who could benefit from immunotherapy.

**Methods:** Single case study and review of the literature

**Case summary and intervention:** We discuss a case of a 26-year-old man with a metastatic osteosarcoma not responsive to several lines of standard chemotherapy. NGS-analysis of resected tumor tissue revealed amplification of PD-L1 and PD-L2, which is associated in literature with response to anti-PD-1/PD-L1 blockade in other tumor types and in osteosarcoma mouse models. Treatment of our patient with the combination of an anti-PD-1 antibody (Nivolumab) and an anti-CTLA4 antibody (Ipilimumab) showed a stabilization of life-threatening retrocardiac tumoral masses that had previously significantly progressed despite radiotherapy, while one bone lesion in the right os ilium was growing and needed treatment with concomitant radiotherapy.

**Conclusion:** To our knowledge, this is the first case report of a metastasized osteosarcoma patient with no further standard treatment options being treated with dual checkpoint inhibition. A complete stabilization of life-threatening retrocardiac lesions could be achieved and with the addition of radiotherapy to a growing bone lesion, the patient is still doing well. NGS-analysis can help identify druggable targets in patients with rare tumors with limited treatment options.

**Abbreviations:** PD-L1: Programmed death-ligand 1; anti-PD-L1: anti-programmed death-ligand 1; PD-1: programmed cell death protein 1; anti-PD-1: anti-programmed cell death protein 1; anti-CTLA4: anti-cytotoxic T-lymphocyte-associated protein 4; AUC: area under the curve; NGS: next-generation sequencing; Gy: Gray; CT scan: computed tomography scan; RCC: renal cell carcinoma; IHC: immunohistochemistry; TMB: tumor mutational burden; NSCLC: Non-small cell lung cancer.

### KEYWORDS

Osteosarcoma; PD-L1 amplification; nivolumab; ipilimumab; immunotherapy

### Introduction/background

Osteosarcoma is the most frequent primary malignancy of the bone and affects mostly children and adolescents but has a second incidence peak in adults older than 60 years. It is a very rare cancer type and counts for less than 1% of all cancer diagnoses [1]. Patients with unresectable metastatic osteosarcoma have a poor prognosis with a 5-year overall survival of less than 25% [2]. The most common metastatic sites are the lungs and bone. Treatment for metastatic disease has hardly improved over the last 20–30 years. In oligometastatic disease, surgery is the only treatment that may enhance overall survival [3]. In case of unresectable metastatic disease, chemotherapy represents the mainstay of therapeutic options but mostly offers only short-term effect [2,3]. New treatment options are therefore urgently needed.

Here, we present a case of a 26-year-old man with a heavily pre-treated lung- and bone metastatic osteosarcoma with no more standard (chemo)therapeutic options. Based upon genomic profiling using next-generation sequencing (NGS) performed on tumor tissue of the last resected pericardial lung metastasis, a PD-L1 and PD-L2 amplification was found. Preliminary data suggest that these findings support the use of checkpoint inhibitors, regardless of the tumor mutational burden and the microsatellite status [4]. Our patient was treated with dual checkpoint inhibition (anti-PD-1 antibody Nivolumab and anti-CTLA4, Ipilimumab) for four cycles, followed by anti-PD-1 inhibition monotherapy as maintenance therapy. A stabilization of the life-threatening retrocardiac masses was obtained. Since the diagnosis of metastatic disease, this was the best observed response to treatment.

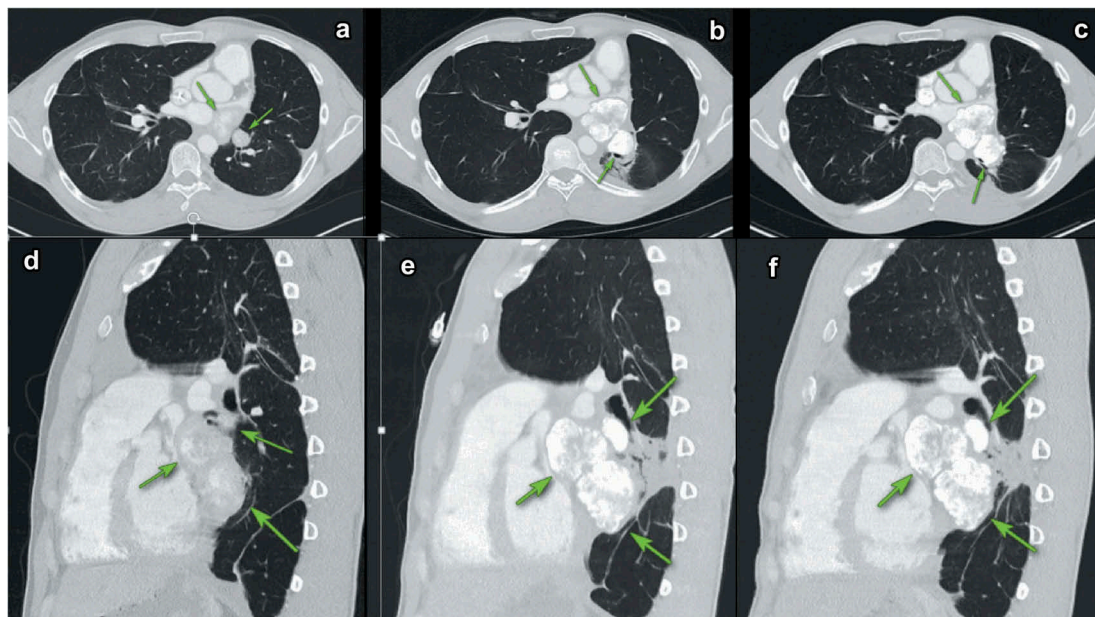
## Case report

In September 2015, a 22-year-old man presented with persistent pain of the left knee and a swelling of the upper leg in a local hospital. Imaging suggested a bone tumor of the left distal femur with an extra-osseous component. He was referred to the sarcoma team of a Belgian university hospital for further diagnosis. Pathology obtained by open biopsy showed an osteosarcoma of the telangiectatic subtype. At the time of diagnosis, there was no evidence of distant metastases. The patient received neoadjuvant chemotherapy, which consisted of cisplatin 100 mg/m<sup>2</sup> on day 1, and doxorubicin 25 mg/m<sup>2</sup> on day 1–3 every 3 weeks. After three cycles, the patient underwent a wide distal femoral transarticular resection and prosthetic reconstruction. The resection margins were macro- and microscopically tumor-free. The longest diameter of the resected tumor measured 11 cm. The pathology report showed tumor necrosis (70%) at the center of the tumor, while the extra-osseous tumor tissue was clearly viable, indicating poor treatment response. One month after surgery, chemotherapy was continued in adjuvant setting for another three cycles until February 2016.

In October 2016, 8 months after finishing the curative treatment, a follow-up scan detected multiple new calcified nodular lung lesions (three in the right lower lobe and five in the left upper lobe). The largest lesions measured 17 mm. Due to practical reasons, he was referred to our specialised sarcoma team at the Ghent university hospital for further treatment. We started him on first-line treatment for metastatic disease: high dose methotrexate 12 g/m<sup>2</sup> on day 1 and 8, and ifosfamide 9 g/m<sup>2</sup> every cycle divided over 3 days (day 22–24). After two cycles of chemotherapy, the lung lesions remained stable and metastasectomy of all suspicious nodules was performed in January and February 2017. Histology of the resected lung metastases showed complete resection of all lesions with no involvement of the pleura and confirmed the diagnosis of metastases of the known osteosarcoma. Postoperative scans showed no evidence of disease. Two months after this last surgery new bilateral lung lesions were diagnosed on CT scan for which new systemic treatment with carboplatin (AUC 5) on day 1 and etoposide phosphate 100 mg/m<sup>2</sup> on day 1, 2 and 3 every 3 weeks was started in May 2017. After 2 cycles two lesions had significantly grown, one of which was located in the right lung very close to the pericardium and a second peribronchovascular lesion in the left lung. We proposed to switch chemotherapy to Gemcitabine 900 mg/m<sup>2</sup> on day 1 and 8 and docetaxel 100 mg/m<sup>2</sup> on day 8. After 2 cycles of this regimen, the same two lesions showed progression, with the right pericardial lesions now measuring 50 mm in longest diameter. As pericardial involvement was suspected on CT scan, radiotherapy was not feasible. In August 2017

and after discussion at the Sarcoma Multidisciplinary Meeting, the right pericardial lesion was resected en bloc and a partial pericardectomy with pericardial patch was performed as tumor invasion was confirmed during surgery. At that time, the lesion in the left lower lobe was the only remaining metastasis. Three months later on November 2017, new imaging showed progression of this lesion, measuring 39 × 39 mm with formation of a large tumor thrombus in the pulmonary vein continuing into the left atrium. The patient therefore underwent an urgent resection with successful removal of the tumor thrombus. After resection of this last lesion, no evidence of disease was reported. Nine months after the last surgery, in August 2018, a new large retrocardiac mass had formed in the left lung close to the aorta, left atrium and ventricle, slightly compressing the left pulmonary vein. This mass consisted of two large calcified lesions measuring 36 × 27 mm and 37 × 31 mm, respectively, and one peribronchial nodule measuring 18 × 17 mm (Figure 1). In addition, a new calcified nodule next to the left bronchus (20 mm) and a small pulmonary nodule (paravertebral right, 4 mm) were described. Considering their location close to critical structures, the risk of life-threatening complications with further growth of the large masses was considered very high. New surgical resection was estimated as too high a risk in this non-curative setting. Radiotherapy of the largest and most life-threatening retrocardiac masses was feasible and a total of 45 Gy (15 × 3 Gy) was given. In the absence of promising systemic treatment options, we proposed screening for ongoing phase I and II trials but he was not eligible for any running trial due to the rare tumor type and lack of necessary driver mutations. At the same time, NGS-analysis of the last resected tumor tissue was performed using the Foundation Heme assay (Foundation Medicine, Cambridge, MA). This showed amplification of PD-L1 and PD-L2. The tumor appeared to be microsatellite stable and tumor mutational burden was intermediate (seven mutations/megabase). Other findings were: FANCE and JAK2-DERL3 rearrangement (intron 3), IKZF2 deletion (exon3) and amplification of MAPK1, AURKB, BCL2L2, CCND3, FGF10, IGF1R, JAK2, KDM4C. Based on these findings and supporting literature [5–7], Bristol-Myers Squibb (BMS) provided Nivolumab and Ipilimumab for this patient through a medical need program. This option was discussed with the patient and his family. He gave his oral consent to start the experimental treatment. We planned four cycles of nivolumab 3 mg/kg and ipilimumab 1 mg/kg every 3 weeks, followed by nivolumab 3 mg/kg every 2 weeks. This treatment was initiated in January 2019, 4 months after completion of radiotherapy. In the meantime and despite radiotherapy, the retrocardiac masses had all progressed and a new bone lesion with cortical breakthrough, bone destruction, and soft-tissue





**Figure 1.** Axial CT scan of de retrocardiac mass in the left lung in August 2018 (a), December 2018 (b), May 2019 (c). Sagittal CT scan of the retrocardiac mass in the left lung in August 2018 (d), December 2018 (e), May 2019 (f).

In Augustus 2018, the two calcified retrocardiac lesions measured  $36 \times 27$  mm and  $37 \times 31$  mm, the peribronchial-calcified nodule  $18 \times 17$  mm. In December 2018 they all progressed and measured now, respectively,  $40 \times 33$  mm,  $41 \times 34$  mm and  $29 \times 28$  mm. These measurements stayed unchanged in May 2019.

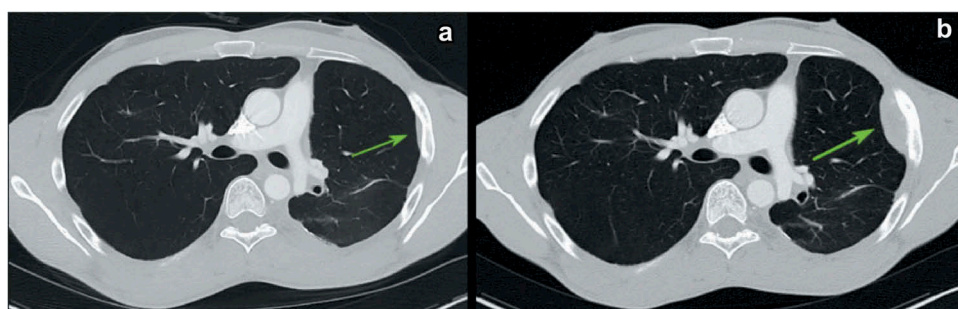
extension had developed in the right acetabulum. The two calcified retrocardiac lesions now measured  $40 \times 33$  mm (originally  $36 \times 27$  mm) and  $41 \times 34$  mm (originally  $37 \times 31$  mm) and the peribronchial calcified nodule  $29 \times 28$  mm (originally  $18 \times 17$  mm) (Figure 1)

The first evaluation after three infusions of the combination nivolumab and ipilimumab showed stable dimensions of the multiple lung masses (Figure 1). The bone metastasis at the right acetabulum, however, had increased in volume (Figure 2). As the patient reported increasing pain in his right hip, we referred him for palliative radiotherapy (15 fractions of 3 Gy) while safely

continuing the systemic treatment. As planned, nivolumab monotherapy was continued every 2 weeks after four cycles of dual checkpoint inhibition. After another 9 weeks of treatment, the lung lesions all remained stable. Especially, the large life-threatening masses had not changed. The soft-tissue component of the acetabulum metastasis showed oedema due to the recent radiotherapy treatment (Figure 2). Unfortunately, a new suspicious pleural thickening had emerged originating from scar tissue of previous repeated thoracotomies, measuring  $19 \times 45$  mm (Figure 3). At that time, the patient had an excellent performance status (WHO 0) and was still



**Figure 2.** CT scan of the bone metastasis in the right os ilium in December 2018 (a), March 2019 (b) and May 2019 (c).



**Figure 3.** CT scan of the pleural metastasis in March 2019 (a) and May 2019 (b).

working. The pain in the right hip had decreased significantly following radiotherapy and he had no more need for pain medication. After discussion on our multidisciplinary meeting, local radiotherapy of the pleural metastasis was proposed while continuing Nivolumab.

## Discussion

Osteosarcomas are rare tumors and treatment options are limited, specifically in a metastatic setting. In the past years, immunotherapy has proven to be a valuable asset in the treatment of different cancer types. In contrast, the clinical data of immunotherapy in osteosarcoma are very limited and whether or not it holds promise as a putative treatment option in the future remains unclear.

We presented a patient with a heavily pretreated metastatic osteosarcoma with life-threatening retrocardiac masses and no more standard treatment options. Based upon NGS-analysis showing PD-L1 and PD-L2 amplification, the patient was treated with dual checkpoint inhibition. The dosages were the same as used for untreated intermediate- and poor-risk advanced RCC [8]. Up till now, the retrocardiac masses are under control, which was our main concern due to their localisation close to the heart and critical vascular structures. Given that at the time of the start of treatment with checkpoint inhibitors the lung masses had significantly progressed despite radiotherapy, this result is remarkable and should be considered a significant clinical effect. As the large retrocardiac lung lesions are highly calcified, shrinkage of these lesions was not to be expected (Figure 1). At present, the patient is still alive with an excellent quality of life. In this case, NGS-analysis led to the identification of checkpoint inhibition as possible treatment option influencing his prognosis in a positive manner.

A crucial crosstalk between bone cells and the immune system has been reported in preliminary studies, suggesting that the immune system would be a possible therapeutic target in the treatment of malignant bone tumors. Currently multiple immune-targeted therapies are being investigated in the advanced and metastatic setting, including the use of checkpoint inhibitors [9]. At the current time, the clinical evidence of immunotherapy for the treatment of osteosarcoma is limited to preclinical data and one phase II trial in sarcoma patients. In K7M2 mouse models of metastatic osteosarcoma, blockade of PD-1/PD-L1 resulted in decreased tumor burden and increased survival [10]. Eventually, tumors in these mouse models became resistant to anti-PD-1 by upregulation of inhibitory receptors on tumor-infiltrating lymphocytes, including CTLA-4 [5]. The combination of PD-1 and CTLA-4 inhibitors in these mice appeared to have a synergistic effect and complete responses were observed. Similar findings were originally also described in B16 mouse model of metastatic melanoma [11]. In melanoma, dual checkpoint inhibition is now part of the standard of care as it showed higher

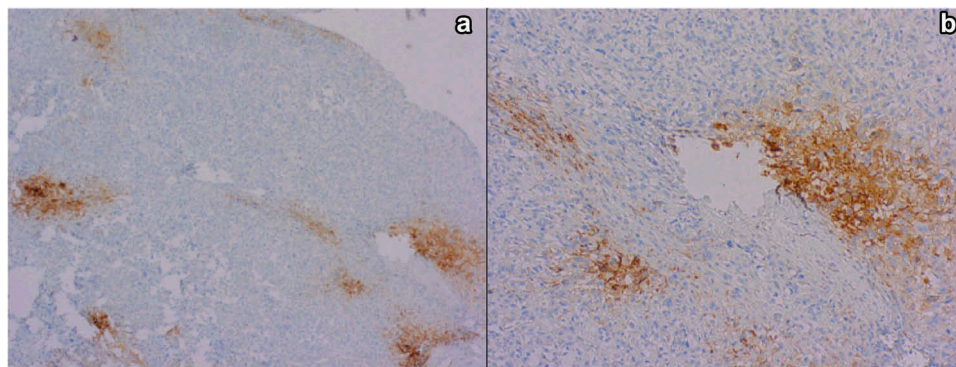
objective response rates compared to PD-1 blockade alone in a phase III trial [7]. In osteosarcoma, on the other hand, no reports or trials have been published treating patients with the combination of anti-CTLA4 and anti-PD-1 therapy. The only data available were generated during a phase 2 open-label, single-arm trial investigating the activity of pembrolizumab (an anti-PD-1 antibody) in soft-tissue and bone sarcoma. Of the 86 patients enrolled, 22 patients had osteosarcoma. Six of these osteosarcoma patients (27%) had stable disease and 1 patient (5%) showed a substantial tumor volume reduction and durable response (>6 months) upon anti-PD-1 treatment. Currently, multiple trials are ongoing investigating the anti-tumor activity of anti-PD-1 with or without anti-CTLA4 or other active components in (osteo)sarcoma. (registered at [www.ClinicalTrials.gov](http://www.ClinicalTrials.gov) – NCT02500797, NCT02304458, NCT02500797) As is often the case in sarcoma studies, most of these trials include all subtypes of sarcoma, which makes the results challenging for interpretation.

Despite the remarkable results in melanoma, other tumor types showed less significant response rates to checkpoint inhibition. This has led to the suggestion for the use of biomarkers to select patients with high response rates to these expensive treatments. Evidence originates from various clinical studies. For example, in patients with advanced NSCLC treated with Nivolumab or Pembrolizumab, response rates correlated positively with PD-L1 expression on tumor cells [12]. This has led to the EMA approval of Pembrolizumab in first-line treatment of metastatic NSCLC in adults with tumoral PD-L1 expression of >50%.

However, there are several limitations to these studies: usage of different thresholds denominating positive PD-L1 expression, PD-L1 staining on different cells, poor uniformity in the antibodies used in IHC analysis, patient variability, poor to modest negative predictive value. This explains why PD-L1 testing alone is insufficient for patient selection in most malignancies [13,14]. In our patient, PD-L1 expression was present in 15–20% of (viable) tumor cells, which is considered high and suggests that in this case, it could be a biomarker for response (Figure 4). However, in the abovementioned phase 2 trial in sarcoma patients, only 4% of the total population had a positive PD-L1 expression (defined as a positive staining of >1%) and none of those had an osteosarcoma [6]. It is clear that the role of PD-L1 expression in osteosarcoma has not been clarified. High expression of PD-L1 is frequently seen in osteosarcoma, but correlation with response to checkpoint inhibition is not known [15,16].

Our decision to treat this young man with immunotherapy was not based on PD-L1 expression detected on IHC but on NGS analysis showing PD-L1 and PD-L2 amplification. In general, the prevalence of PD-L1 amplification in solid tumors is rare [4]. Although PD-L1 and PD-L2 amplification is less intensively investigated as PD-L1 expression, a large retrospective study showed that PD-L1





**Figure 4.** PD-L1 staining on tumor tissue of resected lung metastasis, magnification 40× (a) and 100× (b).

amplification was associated with response to checkpoint inhibition even in the absence of microsatellite instability, high PD-L1 expression or high tumor mutational burden [4,13]. These tumor responses were frequently durable. PD-L1 amplification has therefore been suggested as predictive biomarker [4,13]. Other suggested predictive biomarkers that are associated with an increased likelihood of response are microsatellite instability (MSI) and high tumor mutational burden (TMB) [13,17]. Not much is known about MSI status and TMB in osteosarcoma. The frequency of MSI in osteosarcoma has not been described [18]. Although genetic aberrations are frequently seen in osteosarcoma, TMB is often low to moderate. It is not known whether the tumor-specific neoantigens that are produced have the ability to stimulate the immune system and therefore will predict response to anti-PD-1/PD-L1 treatment [18–20]. In our case, the tumor was microsatellite stable and TMB was intermediate (seven mutations/megabase), indicating that these markers do not have a predictive value in this patient.

During the treatment with checkpoint inhibition, we observed a mixed response in the different metastatic sites. The retrocardiac masses stabilized, while a bone lesion and a pleural mass showed progression. It has been reported that PD-L1 expression can vary between different tumor sites in the same patient [21]. Metastases of the lung more frequently express higher PD-L1 levels versus other sites. This is probably related to the specific lung microenvironment or to the evolution of the tumor clones in time as lung lesions often present earlier in the disease course of sarcomas [16]. As the decision to treat our patient with immunotherapy was already made upon the results of the NGS-analysis, IHC for PD-L1 expression was not performed until treatment was already started. The tissue used for PD-L1 staining in our patient was the same sample used for NGS-analysis and originated from the pericardial mass resected in August 2017. The high PD-L1 expression of 15–20% of tumor cells matches the observed stabilization of the retrocardiac masses in our patient upon treatment with dual checkpoint inhibition (Figure 4). Interestingly, the origin of the tumor tissue sample used for NGS-analysis can also explain the mixed response we observed in our patient. The lung metastases,

responding to dual checkpoint inhibition, most likely originate from the same clone as the analyzed tissue given their proximity, whereas the bone lesion in the right hip and the left pleural mass probably represent a clone with a different biology and PD-L1 expression. As a biopsy of those lesions was not performed (given the non-curative setting), this hypothesis can not be confirmed but it raises the question on how NGS-analysis could best be used in cancer patients with metastatic disease.

It has been suggested that NGS analysis in sarcoma can identify potentially targetable genomic alterations, copy number alterations and gene fusions in rare tumors [22–24]. The biologic diversity and high mutation rates in sarcomas make them an interesting subject for personalized medicine. There are multiple reports of NGS-based molecular profiling in sarcomas investigating their clinical utility [23]. So far, only case reports have been published showing remarkable therapeutic efficacy following molecular profiling [22,23]. It is to be expected that the number of patients who have actionable NGS findings will increase as more molecular targeted therapies are developed and the use of molecular profiling becomes more accessible [22]. With our case report, we support the use of NGS in rare cancers as this will help to improve our insights in the biology of rare cancers and will identify new potential treatment options in young, fit patients with no therapeutic options left.

## Conclusion

In rare cancers, progress in treatment development is disappointing and access to promising trials is limited. This case report showed an interesting role of NGS analysis in a patient with a rare cancer and limited treatment options in finding PD-L1 and PD-L2 amplification. However, the question remains which tumoral lesion should be selected for NGS analysis and what is the perfect timing in the disease course.

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## Author's contribution

All authors read and approved the final manuscript.

## Consent for publication

Consent form for publication signed by the patient is available.

## Disclosure statement

No potential conflict of interest was reported by the authors.

## ORCID

Laure Nuytemans  <http://orcid.org/0000-0003-2116-7136>

Gwen Sys  <http://orcid.org/0000-0002-5308-7846>

David Creyten  <http://orcid.org/0000-0002-6064-1673>

Lore Lapeire  <http://orcid.org/0000-0002-5028-6305>

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