TILs in Head and Neck Cancer: Ready for Clinical Implementation and Why (Not)?

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Abstract The assessment of tumor infiltrating lymphocytes (TILs) has recently emerged as a prognostic biomarker in several solid tumors. Quantification and subtyping of TILs reflects the immune response in the tumor microenvironment, contributing to either tumoral immune attack or escape and thereby affecting outcome. Despite the growing evidence of its value as prognosticator, TILs analysis has not yet found its way to daily clinical practice. The aim of this review is to evaluate whether the current knowledge on TILs in head and neck cancer justifies its clinical implementation. Therefore, we summarize the data on TILs in squamous cell cancer of the head and neck with focus on the most important subsets (T lymphocytes and more specifically CD8⁺ cytotoxic T cells and FoxP3⁺ regulatory T cells) and site-specific characteristics such as Human Papilloma Virus infection. In addition, we discuss methodological problems and pitfalls that can account for discordant findings and that may hamper inclusion of TILs assessment in routine practice of pathologists and oncologists.

Keywords Squamous cell carcinoma of the head and neck · Tumor infiltrating lymphocytes · Prognosis · Biomarker

Introduction

Head and neck cancer is the sixth most common malignancy in the world, accounting for 550,000 new cases every year [1] and is a term used to group together a number of tumors arising from distinct subsites including nasal cavity, oral cavity, pharynx and larynx. Its association with tobacco and alcohol has been investigated and confirmed in various trials [2, 3]. More recent, a growing number of cases of SCCHN has been linked to the Human Papilloma Virus (HPV), as this virus is involved in 30–65% of all SCCHN and up to 80% of oropharyngeal cancers [4–7].

Current treatment regimens for SCCHN rely on a combination of surgery, radiotherapy and systemic therapy, depending on the tumor stage [8, 9].

Outcome for locoregional advanced disease (stage III/IV) remains dismal due to local recurrence and/or distant metastasis. In Europe the relative survival rate for head and neck cancer patients is 72% at one year and 42% at five years [8]. This urges the need for more effective treatments with fewer side effects. In this context, targeting the tumor microenvironment with immunotherapy may present a potential approach for the control of head and neck cancer.

The tumor microenvironment is a unique and complex environment that emerges during the course of tumor progression [10]. It consists of fibroblasts, stroma and immune cells, including those mediating adaptive immunity as well as effectors of innate immunity. T lymphocytes, including CD3⁺CD4⁺ and CD3⁺CD8⁺ T cells, are the predominant component of the tumor microenvironment in solid tumors [10, 11]. In addition to T cells, macrophages (M1 and M2 macrophages), natural killer (NK) cells, and dendritic cells (DCs) also infiltrate tumor tissue [10–13]. Research has shown that CD4⁺ T helper cells, CD8⁺ cytotoxic T cells, NK cells,

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M1 macrophages and DCs are protective against tumor growth and that a coordinated and balanced interaction of these subsets is needed to protect the host against a developing tumor [14]. Conversely, $CD4^+$ forkhead box P3⁺ (FoxP3⁺) regulatory T cells (T_{reg} s) and myeloid-derived suppressor cells (MDSCs) promote tumor growth [14].

T_{reg} s, which are characterized by the presence of FoxP3, involved in the development and function of these cells, play a key role in the process of immune-escape by producing the immunosuppressive cytokines IL-10 and TGF- β , and by consuming IL-2. Consequently, T_{reg} s express the negative co-stimulatory checkpoint inhibitors such as CTLA-4 and PD-1, which dampen T-cell activity when bound to their respective ligands [15–17]. The process of immune-escape is further regulated by MDSCs which inhibit lymphocyte function by inducing T_{reg} cells, producing TGF- β , depleting or sequestering the amino acids required for T cell function, or nitrating T cell receptors or chemokine receptors on tumor-specific T cells [18, 19].

Figure 1 is a simplified schema of the interaction between tumor and immune cells.

Next, a dynamic interaction takes place between immune cells and the tumor itself, called immunoediting [10]. The process of immunoediting reflects the dual role of the immune system in promoting host protection against cancer and facilitating tumor escape from immune destruction. Those cancer clones that are able to escape the immune system have a survival advantage. Based on the interaction between tumor and immune cells, there are three phases in the process of immunoediting: (1) elimination where cancerous cells are eliminated following immunosurveillance, (2) equilibrium where transformed cells are held in control but are no longer eliminated and (3) escape where tumor cell modifications result in loss of immune response and shape disease progression [10, 11].

In recent years it has become clear that assessment of the immune infiltrate can be of prognostic value in various tumors. In colon cancer, for example, the immunoscore, based on quantification of different types of immune cells, proved to be of greater prognostic significance than conventional TNM staging [20]. Moreover, in the era of immunotherapy, the immune infiltrate of tumors is speculated to be a possible predictive marker of treatment efficacy.

In this review we focus on SCCHN and the available literature on the above mentioned topics. The prognostic role of TILs in SCCHN seems intriguing and will be discussed. Moreover, clinical relevance and the role of TILs in the development of more precise and effective therapies will be highlighted.

Fig. 1 Simplified schema of the interaction between tumor and immune cells. $CD4^+$ (Th1) T cells produce IFN γ , which in turn mediates the expansion, differentiation, and activation of tumor-specific $CD8^+$ T cells. As a result of the complex interaction between the tumor and cytotoxic T cells (TCR-MHC I/ peptide), high levels of granzymes and perforin are released from these cells, resulting in granule exocytosis and tumor cell death via apoptosis. Both FoxP3⁺ T_{reg} cells and MDSCs play a critical role during the selection of high-avidity $CD8^+$ T cells, reducing the functionality of $CD8^+$ T cells. IFN γ interferon gamma, MDSC myeloid-derived suppressor cell, MHC I major histocompatibility complex I, TCR T cell receptor

Tumor Infiltrating Lymphocytes and Outcome in SCCHN

CD3⁺ Tumor Infiltrating Lymphocytes

The immune infiltrate of tumors is mainly composed of lymphocytes where T lymphocytes are the predominant cell type. CD3 is a highly specific marker for these T cells. Bound by its antigen, presented in the context of a major histocompatibility complex molecule, the T cell receptor CD3 complex transduces the activating signals to the cytoplasm of the T cell. Given its high specificity and the fact that it is present on T cells in all stages of their development, CD3 is an interesting marker for the detection of T lymphocytes. In SCCHN, high CD3 expression is more common in tumors arising from the oropharynx. Also,

patients with early T stage tumors and a lack of smoking history are characterized by high number of CD3⁺ TILs [21]. Survival analysis demonstrated that SCCHN patients with high CD3 expression, and thus high number of TILs, had a significant superior overall survival (OS), local progression free survival (LPFS) and distant metastasis free survival (DMFS) in univariate analysis. In multivariate analysis, a high number of TILs showed only a benefit for OS [21, 22]. In agreement to these findings, strong tumor infiltration by CD3⁺ TILs has been correlated with favorable outcome in several tumors [23].

CD8⁺ Tumor Infiltrating Lymphocytes

CD8 positive T lymphocytes are also called cytotoxic T cells and are able to kill infected, neoplastic, or otherwise targeted cells by direct lysis. They are the most powerful anticancer cells of the immune system. High numbers of tumor infiltrating CD8⁺ T lymphocytes are associated with oropharyngeal localization, limited tumor growth and lower incidence of smoking [21, 24]. Patients with lower CD8⁺ T cell count presented a more aggressive disease with higher nodal stage (N_{2c-3} stage) [22, 24]. Interestingly, strong CD8⁺ T cell infiltration was also observed in patients with better patient Karnofsky performance score [25]. In general, cytotoxic T cells are positively associated with favorable outcome as described in other cancers such as colorectal and ovarian cancer [20]. Consequently, patients diagnosed with SCCHN whose tumor was highly infiltrated by CD8⁺ T cells demonstrated a significant better outcome [21, 22, 25–30] compared to minimal or no infiltration. However, in tumors arising from the oral cavity, CD8⁺ TILs had no significant prognostic value [30–32]. In this matter, Wolf et al. [33] detected that higher CD8 infiltrate counts in oral cavity tumors were associated with tumor recurrence. From these results, we can speculate that tumors arising from the oral cavity could have a different biological behavior from other tumors arising from head and neck. This hypothesis is supported by the fact that tumors arising from the oral cavity are characterized by a high degree of local invasion and a high rate of metastasis to the cervical lymph nodes [34]. Interestingly, the prognostic value of CD8⁺ TIL levels also seems to vary between tumor compartments [22, 35–38]. *In concreto*, high CD8 expression in tumor periphery was significantly correlated with improved PFS and local failure free survival whereas high CD8 expression within the tumor cell mass was associated with better OS and DMFS. High CD8 expression in stroma was prognostic for all four clinical end points. In contrast, Balermipas et al. [21] did not report any tumor compartment-dependent differences in the prognostic value of CD8. These opposing results are suggestive for further research to determine

the relevance of different subtypes of TILs according to the tumor compartment.

Taken together, the current data on CD8⁺ TILs and prognosis are not unequivocal and warrant further research. Differences in biological behavior dependent on the anatomic subsite or compartment of the tumor microenvironment may explain the somewhat contradictory findings, as we will discuss below.

CD4⁺ Tumor Infiltrating Lymphocytes

The benefit of CD4⁺ T cell infiltration in tumors is somewhat controversial. Firstly, CD4⁺ T cells were found to be an independent favorable prognostic factor in pancreatic and esophageal squamous cell carcinoma. The same finding was true for SCCHN as high levels of tumor infiltrating CD4⁺CD69⁺ T cells in these patients were positively correlated with OS and locoregional control [32]. In contrast, others have described CD4 to be a predictor of poor outcome, especially in oral cavity tumors [39]. These contradictory results could be explained by the heterogeneity of the CD4⁺ T cell population as besides the classical T helper 1 and T helper 2 cells, other subsets have been identified; including T helper 17, follicular helper T cell, T helper 9, and T_{regs}; each with its proper characteristic cytokine profile [40]. A further definition of the role and distribution of these subsets in SCCHN is therefore needed.

One of these CD4⁺ T cell subpopulations, namely FoxP3⁺ T_{regs}, is the topic of growing interest and has already been examined closely in SCCHN. As stated before, T_{regs} play a key role in the process of immune-escape. A high number of FoxP3⁺ T cells was significantly associated with the primary tumor site: tumors arising from the oral cavity and oropharynx were heavily infiltrated by FoxP3⁺ T cells [41]. High levels of FoxP3⁺ T cells also correlated with good pathological differentiation and early T staged SCCHN [33, 42]. Furthermore, patients with high levels of FoxP3⁺ T cells had better outcome as described already in patients with colorectal cancer [32, 41, 43, 44]. However, Saito et al. [45] recently showed that a low level of FoxP3 expression does relate to a better overall survival in colorectal cancer. These interesting results require further validation and might also be true in SCCHN. On the other hand, in most other tumors the presence of FoxP3⁺ T_{regs} was associated with a poor outcome, as was the case in tumors arising from the oral cavity [31, 42, 46–55]. This finding supports the theory that the tumor-infiltrating FoxP3⁺ Tregs could be an escape mechanism of human cancers to the immune response. The role of T_{regs} in the control of tumor growth remains a topic of controversy in SCCHN and demands further research on the mechanism(s) by which FoxP3⁺ T_{regs} affect outcome.

Influence of HPV Status on TILs and Outcome

Over the past years, the number of cases of SCCHN caused by HPV has increased [4–7]. Although HPV⁺ tumors are often diagnosed in a more advanced stage, studies report significantly better outcome compared to patients diagnosed with HPV[−] tumors. The reason for this advantage is however unclear. Hypothetically, the difference in survival between HPV⁺ and HPV[−] tumors is probably due to a different immune response directed against the viral antigens. Indeed, HPV⁺ tumor samples have significantly higher numbers of infiltrating CD4⁺ and CD8⁺ T cells [26, 27, 44, 56]. Researchers reported that a high CD8⁺ T cell infiltrate was positively correlated with a good clinical outcome in both HPV⁺ and HPV[−] tumors [21, 26, 27], whereas others failed to reproduce this finding [22, 25, 44, 57, 58]. Furthermore, Ward et al. [44] reported that the prognostic significance for high counts of TILs was only valid in HPV⁺ tumors, while others reported a prognostic benefit in HPV[−] tumors only. Hence, the prognostic significance of TILs according to the HPV status of the tumor remains a subject of controversy. More trials, preferably randomized, with a sufficient number of patients are required in order to elucidate the relationship between TILs and the presence of HPV.

Influence of PD-L1 Expression on TILs and Outcome

The programmed death ligand-1/programmed death-1 (PD-L1/PD-1) pathway is known to be involved in T cell activation and differentiation. It has become the focus of attention in oncology, since therapeutic benefits were discovered by blocking either the ligand (PD-L1) or its receptor [59, 60]. To date only little is known on the expression of PD-L1 in SCCHN and most definitely not on its relation with TILs. PD-L1 expression was inversely correlated with the density of intratumoral CD8⁺ T lymphocytes in patients with oral squamous cell carcinoma [24]. Conversely, there was a positive association between the amount of TILs and PD-L1 expression in laryngeal cancer [61]. In this report, it was also shown that increased PD-L1 levels were associated with better outcome in laryngeal squamous cell cancer.

An overview of the described trials conducted in SCCHN is given in Table 1.

Differences in Outcome: What is the Reason?

Studies on the prognostic value of TILs in SCCHN have led to discrepant findings. Several factors might contribute to these differences in prognostic claims and hamper direct comparison of the studies reported in literature.

Biological Factors

Although commonly grouped together, SCCHN comprises malignancies that arise from functionally and anatomically distinct tumor sites with different characteristics e.g. malignancies arising from the oropharynx, which are usually heavily infiltrated by lymphocytes (=pre-existing lymphoid tissue), in contrast to oral cavity tumors. Furthermore, the presence of HPV, which is considered a positive prognosticator, is more often linked to oropharyngeal cancer compared to other tumor sites and as mentioned earlier, TIL infiltration can differ between HPV⁺ and HPV[−] tumors. Recent publications also report a heterogeneous molecular and immunological tumor profile of these malignancies on different anatomical localizations [62]. Differences in prognostic value of TILs might reflect these different biologic factors, making it conceivable that TILs exhibit different properties depending on the tumor site, histologic and/or molecular subtype. As discussed earlier it has been suggested that high infiltration of FoxP3⁺ T_{regs} may inhibit protumoral effects of inflammatory immune cells and may appear as favorable prognostic markers in one tumor site where in other tumor sites the infiltration is linked to poor prognosis due to their conventional regulatory function [32, 41, 44]. Others believe they exhibit their conventional regulatory function in another tumor site where the infiltration of FoxP3⁺ T_{regs} is linked to poor prognosis [31, 42, 46–55].

Additionally, intratumor and intermetastatic heterogeneity complicates this analysis. Table 1 illustrates the use of various samples: biopsies, resection specimens, lymph node metastases. Obviously, a resection specimen of the primary tumor is the ideal study material for a representative assessment of TILs, including their distribution inside the tumor cell mass and in the invasion front of the tumor. However, especially in the field of SCCHN, surgery is not always feasible for different reasons and in these cases, tumor tissue is only available as a small biopsy specimen. It is possible that the immune infiltrate present in the biopsy is not representative for the whole tumor. On the other hand, when limiting a study to resection specimens and excluding cases where only biopsy material can be provided, a selection bias is created towards a group of patients in better general condition and with limited tumor burden, fitted for surgery.

Technical Factors

Different technical aspects of the scoring strategy may have an impact on the quantification of TILs and therefore the outcome of results. Of course, different techniques such as flow cytometry, Western blotting, morphological assessment, or immunohistochemical analysis may yield different results. But for example immunohistochemistry, which

Table 1 Synoptic overview of the effect of TILs on outcome in patients with SCCHN

References	Tumor site	Analytic cohort	Tissue	Phenotyping	Scoring	Key finding
Balermipas et al. [21]	SCCHN	161	Resection	IHC: CD3, CD8	Semi-quantitatively	-High CD3 ⁺ T cell count positively correlates with better OS, LPFS, DMFS -High CD8 ⁺ T cell count positively correlates with better OS, LPFS, DMFS
Balermipas et al. [22]	SCCHN	101	Biopsy	IHC: CD3, CD4, CD8, FoxP3	Semi-quantitatively	-High CD3 ⁺ T cell count positively correlates with better OS -High CD8 ⁺ T cell count positively correlates with better OS, LPFS, DMFS
Nordfors et al. [26]	Oropharynx	275	Biopsy	IHC: CD4, CD8	Quantitatively	-High CD8 ⁺ T cell count positively correlates with better 3Y OS, 3Y DFS in both HPV ⁺ and HPV ⁻ tumors
Wansom et al. [25]	Oropharynx	46	Biopsy	IHC: CD4, CD8, FoxP3	Semi-quantitatively	-CD8, FoxP3 and total T cell counts were positively correlated with OS and DSS -T cell infiltrate was not affected by HPV status
Näsman et al. [27]	Oropharynx	83	Biopsy	IHC: CD8, FoxP3	Quantitatively	-High CD8 ⁺ T cell count positively correlates with better 3Y OS in HPV ⁺ tumors -High number of TILs was positively correlated with HPV ⁺ status
Pretschner et al. [28]	Oropharynx Hypopharynx	33	Resection Lymph nodes Metastases	IHC: CD3, CD8, FoxP3	Quantitatively	-Tumoral CD8 tended towards a favorable DFS
Watanabe et al. [29]	Oral cavity	87	Resection	IHC: CD4, CD8, CD69, FoxP3	Quantitatively	-Low CD8 ⁺ T cell count was inversely correlated with survival
Zancope et al. [30]	Oral cavity	40	Resection	IHC: CD8	Quantitatively	-Peritumoral CD8 ⁺ T cells tended towards a higher mean survival
Tabachnyk et al. [31]	Oral cavity	58	Biopsy Resection	IHC: CD3, CD4, CD8, FoxP3	Quantitatively	-Low FoxP3 ⁺ T cell count positively correlates with NED survival in the post-RCT samples
Badoual et al. [32]	SCCHN	84	Biopsy	Immunofluorescence: CD4 subpopulations	Quantitatively	-High CD4 ⁺ CD69 ⁺ T cell count positively correlates with OS and locoregional control -High CD4 ⁺ FoxP3 ⁺ T cell count positively correlates with better locoregional control

Table 1 (continued)

References	Tumor site	Analytic cohort	Tissue	Phenotyping	Scoring	Key finding
Wolf et al. [33]	Oral cavity	39	Biopsy	IHC: CD4, CD8, FoxP3	Quantitatively	-High CD8 ⁺ T cell count does not correlate with OS or DSS
Ward et al. [44]	Oropharynx	270	Resection Biopsy	IHC: CD3, CD4, CD8, FoxP3	Quantitatively	-High TIL count positively correlates with HPV ⁺ status -High TIL count positively correlates with DSS and PFS in HPV ⁺ tumors
Bron et al. [41]	SCCHN	35	Resection	IHC: FoxP3	Quantitatively	-High FoxP3 ⁺ T cell count positively correlates with better OS
Partlova et al. [56]	Oropharynx	54	Resection Lymph nodes Metastases	Flow cytometry: CD3, CD4, CD8	Quantitatively	-High number of CD8 ⁺ TILs positively correlates with HPV ⁺ status
Sun et al. [49]	SCCHN	83	Resection	IHC: FoxP3	Quantitatively	-Intratumoral presence of FoxP3 ⁺ Tregs is negative prognostic factor
Moreira et al. [39]	Oral cavity	18	Resection	IHC: CD4, CD8, CD25	Quantitatively	-Low CD4 ⁺ T cell count showed a significant increase in survival
Ritta et al. [58]	SCCHN	71	Resection	IHC: CD3, CD4, CD8, FoxP3	Quantitatively	-No difference in the extent of tumor infiltration in HPV ⁺ vs HPV ⁻ tumors
Liang et al. [42]	Oral cavity	81	Resection	Western Blot: FoxP3 IHC: FoxP3	Semi-quantitatively	-Low FoxP3 ⁺ T cell count positively correlates with OS
Cho et al. [24]	Oral cavity	45	Resection	IHC: CD4, CD8	Quantitatively	Intratumoral CD8 ⁺ T cell count inversely correlates with tumoral PD-L1 expression
Vassilakopoulou et al. [61]	Larynx	260	Resection	HE: TILs	Semi-quantitatively	-High number of CD8 ⁺ TILs positively correlates with PD-L1 expression -High density of TILs correlates with better DFS and OS

DMFS distant metastasis free survival, *DSS* disease specific survival, *HPV* Human Papilloma Virus, *IHC* immunohistochemistry, *LPFS* local progression free survival, *NED* no evidence for disease, *OS* overall survival, *PFS* progression free survival, *RCT* radiochemotherapy, *SCCHN* squamous cell carcinoma of the head and neck

is one of the most frequently used methods, may also give various findings depending on the methodology of the test.

Little data have been reported on the different antibodies used for TIL phenotyping. The use of different antibodies can yield different staining patterns because of differences in sensitivity and/ or specificity. Although the effort has been done to compare anti-PDL1 staining antibodies not enough data are available to evaluate the confounding effect of different other antibodies [63, 64].

Next, TILs can reside in tumor cells, stroma or both. Localization may affect the prognostic significance though mixed findings have been reported regarding the impact of the compartment on the prognostic value of TILs. In this context it is also worth mentioning that the tumor and its microenvironment are a dynamic interaction, which questions the relevance of measuring TILs in a somewhat artificial, static manner. On top, it is not clear which region is the most relevant. It has always been assumed that

lymphocytes interacting directly with tumor cells are the most important. However most current studies have found stromal TILs to be a more reproducible parameter. Following the recommendations of Denkert et al. [37] TILs should be scored uniquely as a percentage of the stromal area and areas occupied by carcinoma cells should not be included.

Finally, there is no standard cut-off point for TIL quantification studies at this moment. Results are often based on either mean number of TILs or median number of TILs. Before assessing a valid cut-off value, standardization of the technical issues listed above is of the utmost importance.

Statistical Factors

Correct statistical analyses have to be made in order to fully determine the prognostic value of TILs in SCCHN. Several studies made prognostic claims based on univariate analysis. The major advantages of univariate analysis are the ease of application, ease of interpretation, and ease of communication of the results. However, it is conceivable that the prognostic impact of TILs depends on different other variables including primary tumor site, differentiation and TNM stage. Using multivariate analysis may provide interesting results complementary to the univariate analysis, such as the possible influence of confounders. Importantly, results should be interpreted within the statistical framework (uni- or multivariate) with which they have been produced in order to draw more valid conclusions.

Another drawback in the statistical analysis is the small number of patients studied in some reports. A smaller sample will give a result which may not be sufficiently powered to detect a difference between the groups and the study may turn out to be falsely negative.

Considering these factors leading to inconsistent data, there are still many hurdles to overcome in order to determine the correct prognostic and/or predictive value of TILs in SCCHN and to implement its assessment in the daily practice of pathologists and oncologists. It appears obvious that evaluation of TILs in SCCHN has to be performed in a standardized manner. Prospective studies are warranted to evaluate the prognostic and/or predictive relevance of TILs in SCCHN patients by using standardized methodology and validated cut-off values, well defined treatment strategies, strict follow up schemes and multivariate analyses of clinicopathological variables of the patients.

Current Clinical Relevance of TILs

The presence of TILs in SCCHN opens up important questions for patient management. It is likely that patients with high TILs can benefit from strategies designed to enhance

the immune response against the tumor. This observation has been a major obstacle in the development of new therapeutic approaches since platinum based regimens and radiotherapy have been considered immunosuppressive. However, recent insights have demonstrated that conventional therapies such as chemotherapy and radiotherapy may result in an immunogenic cell death [65, 66]. This could provide an excellent setting for immunotherapeutic strategies to complement current standard chemoradiation. Like in other tumors, combining chemotherapy, radiotherapy and immunotherapy is topic for investigation with the goal to improve disease control and outcome in patients with SCCHN.

Conclusion

In conclusion, we have demonstrated that the prognostic value of different types of TILs in SCCHN may differ among literature. The original view that FoxP3⁺ T_{regs} are associated with poor outcome while CD8⁺ lymphocytes equal good prognosis is oversimplified. Discrepancies in prognostic impact can be attributed to several factors, both technical and biological in nature. Importantly, all of the aforementioned reports published only on the quantification of TILs in SCCHN, while functionality remains elusive and needs to be studied further.

Further improved understanding of the tumor microenvironment in SCCHN is necessary and could allow the use of immune markers as prognostic markers and enable the development of more precise and effective therapies.

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Compliance with Ethical Standards

Conflict of interest Astrid De Meulenaere declares that she has no conflict of interest. Tijl Vermassen declares that he has no conflict of interest. Sandrine Aspeslagh declares that she has no conflict of interest. Katrien Vandecasteele declares that she has no conflict of interest. Sylvie Rottey declares that she has no conflict of interest. Liesbeth Ferdinande declares that she has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

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