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Effector memory T cell frequencies in relation to tumour stage, location and HPV status in HNSCC patients

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Abstract

Background: The immune system plays an important role in tumour immune surveillance. Head and neck squamous cell carcinoma patients are often immune compromised.

Objective: To chart the baseline levels of T cell subpopulation frequencies in patients with cancer prior to treatment.

Subjects and methods: Blood samples of patients were taken at the time of diagnosis, analysed with flow- cytometry and compared with blood samples of healthy donors.

Results: Compared to healthy donors, a significant shift from naive to effector memory T cells was observed. This effect was most prominent in stage II patients. A similar shift from naive to effector memory T cells was noted in patients with oropharynx or larynx squamous cell carcinomas. Furthermore, the percentage of effector memory and effector T cells was higher in the group of patients with human papillomavirus-positive oropharyngeal squamous cell carcinomas, compared with patients with human papillomavirus-negative tumours, suggestive of virus-induced T cell activation.

Conclusion: Here, we provide a simple and easily implementable tool to document T lymphocyte subsets in the peripheral blood of head and neck cancer patients, which might be useful for prognosis and/or therapy response prediction.

Keywords: head and neck cancer; T cell subpopulations; HPV; peripheral blood lymphocytes; prognostic factor

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the 6th most prevalent form of cancer in the world (Moore *et al*, 2000; Kamangar *et al*, 2006). The five-year overall survival for advanced HNSCC patients is about 50% (Matta and Ralhan, 2009). The two main risk factors for the development of HNSCC are tobacco use and excessive alcohol consumption (Brennan *et al*, 1995). A third and increasingly important risk factor consists of persistent infection with human papillomavirus, particularly the high-risk type HPV16 (Klussmann *et al*, 2001; Lajer and von Buchwald, 2010; Leemans *et al*, 2011). Most HPV-positive cancers in the head and neck region are oropharyngeal squamous cell carcinomas (OPSCC). In the United States, the prevalence of HPV-positive OPSCC is estimated at around 70%, where this ranges between 20% and 60% in Europe (Rietbergen *et al*, 2012; Sanders *et al*, 2012). Patients with HPV-positive OPSCC generally have a better prognosis than patients with HPV-negative OPSCC (Fakhry *et al*, 2008; Lassen *et al*, 2009; Ang *et al*, 2010; Dayyani *et al*, 2010; Hong *et al*, 2010).

Patients with HNSCC have reduced numbers of lymphocytes in their blood compared with healthy individuals (Kuss *et al*, 2004). This may be explained by the presence of higher levels of apoptosis markers and spontaneous apoptosis *ex vivo* (Saito *et al*, 1999; Hoffmann *et al*, 2002). In general, cancer treatment, often consisting of surgery followed by radiotherapy or a combination of radiotherapy and chemotherapy, can have a major influence on functionality and viability of peripheral blood lymphocytes. Detrimental effects of treatment on peripheral blood lymphocytes have been observed in other types of cancer, such as breast cancer (Mackay *et al*, 1984), cervical cancer (Bachtiary *et al*, 2005) and glioblastoma multiforme (Fadul *et al*, 2011). Similar to many other tumour types, HNSCC tumours are shown to have immune suppressive effects as demonstrated by the *in vitro* reduction in Th1 cytokines produced by lymphocytes in the presence of HNSCC tumour cell lines, or supernatants derived thereof (Kacani *et al*, 2003).

The immune status and potential immunogenicity of the tumours may explain the relatively good prognosis for patients with HPV-positive HNSCC (Vu *et al*, 2010). In the Netherlands, where the study population of the current study resides, most of the HPV-positive tumours arise from the oropharynx (Braakhuis *et al*, 2004). HPV-negative tumours are characterized molecularly by a high frequency of TP53 mutations, whereas TP53 mutations are rarely seen in HPV-positive squamous cell carcinomas (SCC) (Smeets *et al*, 2006). The number of tumour infiltrating lymphocytes is significantly higher in HPV- positive SCC than in HPV-negative SCC (Fakhry *et al*, 2008; Lassen *et al*, 2009; Ang *et al*, 2010; Dayyani *et al*, 2010). The influence of tumours on the immune system is not restricted to the direct tumour environment, but can also be seen systemically, for example, in peripheral blood. Wansom *et al* showed that HNSCC patients with HPV-positive SCC have higher numbers of $CD8^+$ T cells in the peripheral blood correlating with an improved clinical outcome, suggesting an important role for the adaptive immune system in HPV-positive patients (Wansom *et al*, 2010). This apparent superior immunogenicity may, at least in part, be related to HPV-specific T cell responses (Heusinkveld *et al*, 2012), which may be further enhanced by therapeutic HPV-based vaccines (Wu *et al*, 2011).

Immune profiling is becoming generally recognized as a prominent prognostic and predictive tool in oncology (Ambs *et al*, 2008; Zitvogel *et al*, 2011). Particularly, analyses

of immune infiltrates in tumours and their precursor lesions have been proposed as an alternative classification system of cancer. This immune score may be predictive of clinical outcome, possibly even more accurately than the conventional American Joint Committee on Cancer (AJCC) staging system (Galon *et al*, 2012).

Documenting the presence of T cell subsets in the peripheral blood of patients with HNSCC, at the time of diagnosis, allows for the determination of a peripheral immune score. This score can be correlated with the clinical outcome at the standard five-year evaluation point. Profiling of lymphocyte subsets in peripheral blood presents a minimally invasive tool for therapy management and follow-up in patients with cancer. In the current pilot study, we compared absolute numbers and percentages of circulating T cell subsets in healthy donors and patients with HNSCC. The effects of tumour location and tumour stage were taken in to account for all patients, whereas the presence or absence of HPV was for those patients with oropharyngeal tumours.

Materials and methods

Patients and controls

Patients with HNSCC (n = 83), treated at the VU University Medical Center in Amsterdam, the Netherlands, and age-matched healthy volunteers (referred to as healthy donors/HD) (n = 15) participated in the current study, between February 2010 and May 2011. Peripheral blood samples (500 µL) were obtained by vena puncture at the time of diagnosis before treatment. Small blood samples were available for this pilot study to be used for flow cytometric analyses. All patients signed an informed consent form, approved by the Institutional Review Board. The patient cohort included 53 men and 30 women with a mean age of 63 years (range, 30–86). Healthy controls included nine men and six women with a mean age of 58 years (range, 50–66). The clinico-pathological characteristics of the patient cohort are summarized in Table 1. The 'UICC/ AJCC TNM classification of malignant tumours' 7th edition was used for tumour staging purposes (Sobin *et al*, 2009; van Monsjou *et al*, 2012). Most HPV-positive tumours arise from the oropharynx, and it is therefore standard procedure in our institute that only patients with OPSCC are tested for the presence of HPV. P16 positivity was used as an initial marker for the presence of HPV (Marur *et al*, 2010). Paraffin-embedded material from all oropharynx tumours was obtained via a incisional biopsy, or from the resection specimen, and was stained for p16, followed by an HPV-specific PCR to confirm the presence of the virus in the tumour samples (Dayyani *et al*, 2010).

Table 1 Clinicopathological characteristics of the patients with HNSCC. *The UICC/ AJCC TNM classification of malignant tumours (7th edition) was used for staging (Sobin et al, 2009). A p16 staining on paraffin- embedded tumour material was used on the oropharynx tumours. In case of a positive p16 staining, an HPV-specific PCR was performed to confirm the HPV status*

83 HNSCC patients		No	%
Average age		63	
Gender	Male	53	64%
	Female	30	36%
Stage	Stage I	8	10 %
	Stage II	21	25%
	Stage III	22	27%
	Stage IV	32	39%
Site of Origin	Oral Cavity	25	30%
	Oropharynx	31	37%
	Hypopharynx	12	14%
	Larynx	15	18%
HPV status	HPV-	16	52%
(Oropharynx only)	HPV+	9	29%
	Unknown	6	19%

Flow cytometry

Whole blood samples were used to quantify various types of lymphocytes using fluorescence-labelled antibodies used at optimal concentrations and analysed by 4-colour flow cytometry. Antibodies used were the following: IgG1 FITC (clone X40, used 1:15), IgG1 PE (clone X40, used 1:15), IgG1 APC (clone X40, used 1:30), CD3 FITC (clone SK7, used 1:15), CD3 PE (clone SK7, used 1:10), CD3 PerCP (clone SK7, used 1:10), CD3 APC (clone SK7, used 1:30), CD4 FITC (clone SK3, used 1:15), CD8 PE (clone SK1, used 1:15), CD27 FITC (clone L128, used 1:10), CD45RO PE (clone UCHL1, used 1:10) (BD Biosciences, Heidelberg, Germany), IgG1 RPE-cy5 (clone DAKG01, used 1:10) (Dako, Glostrup, Denmark), CD56 PE (clone MOC-1, used 1:15) (IQProducts, Groningen, the Netherlands) and CD45RA APC (clone HI 100, used 1:10) (eBioscience, San Diego, CA, USA). Whole blood samples were incubated with appropriate antibodies at 4°C for 20 min, after which the erythrocytes were lysed using BD lysing solution (BD Biosciences) according to the manufacturer's protocol. Samples were analysed on FACSCalibur using CELL QUEST PRO software (v 5.2, BD Biosciences). Absolute numbers of $CD45^+$ lymphocytes were quantified using microbeads (Truecount, BD Biosciences).

$CD3^+$ T cell subsets were further characterized on the basis of expression of CD27, CD45RO and CD45RA. We defined the following subpopulations of $CD3^+$ T cells (Hamann *et al*, 1997; Di *et al*, 2011): Naive; $CD27^+$ $CD45RA^+CD45RO^-$, Central Memory; $CD27^+$ $CD45RO^+$ $CD45RA^-$, Effector Memory, $CD27^-CD45RO^+CD45RA^-$ and Effector T cells $CD27^-CD45RO^-CD45RA^+$. CD56 was used as activation marker in T cells that has also been related to HPV-related tumour conditions (Hilders *et al*, 1994; Santin *et al*, 2003) and terminally differentiated cytokine-induced killer cells with high cytolytic potential (Pievani *et al*, 2011; Kelly-Rogers *et al*, 2006).

Statistical analysis

Paired Student's t-tests or one-way ANOVA tests with a Tukey's post-test were used to determine statistical differences when data points showed a Gaussian distribution. In case of a non-Gaussian distribution, the Mann–Whitney U-test or Kruskall–Wallis test was used, followed by Dunn's post-test. Findings were considered statistically significant when P-values were < 0.05. Statistical analyses were performed using GRAPHPAD PRISM [Version 5 (2007), GraphPad Software, Inc., La Jolla, CA, USA].

Results

Age and the number of peripheral T lymphocytes

Negative effects of cancer treatment on the numbers and phenotype of T cells have been reported (Mackay *et al*, 1984; Bachtiary *et al*, 2005; Fadul *et al*, 2011). Here, we obtained small blood samples from patients at the time of diagnosis, circumventing any effects of treatment on T cells. In elderly people in general, the thymic output is reduced, leading to a reduction in T cell production (Derhovanessian *et al*, 2008). Age range, means and medians in years for healthy donors (HD) and patients in the current study were as follows: HD range: 50–66, mean: 58, median: 58 (N = 15) and HNSCC patients' range: 30–86, mean: 63 and median: 62 (N = 83). Figure 1 shows the number of $CD3^+$ T cells in peripheral blood vs age of all patients with HNSCC and healthy donors. In patients as well as in HD, a clear decline was noted. The absolute numbers of $CD8^+$ T cells remained near constant in the peripheral blood of patients with HNSCC as well as in HD Hence, the decline found in absolute numbers of $CD3^+$ T cells in patients and HD was mainly caused by a decrease in the number of $CD4^+$ T cells.

Absolute numbers of peripheral T cell lymphocytes and tumour stage

The absolute numbers of circulating $CD3^+$ T cells in patients with HNSCC and HD are shown in Figure 2. Patients with HNSCC had significantly lower numbers of circulating $CD3^+$ T cells compared with HD (ANOVA, Tukey's post-test $P < 0.05$). This was observed in early stage patients (stage I + II) ($P < 0.05$) as well as in advanced staged patients (stage III + IV) ($P < 0.05$). No significant differences in absolute numbers of $CD4^+$ ($P = 0.63$) or $CD8^+$ ($P = 0.91$) T cells were observed between early and advanced stage HNSCC.

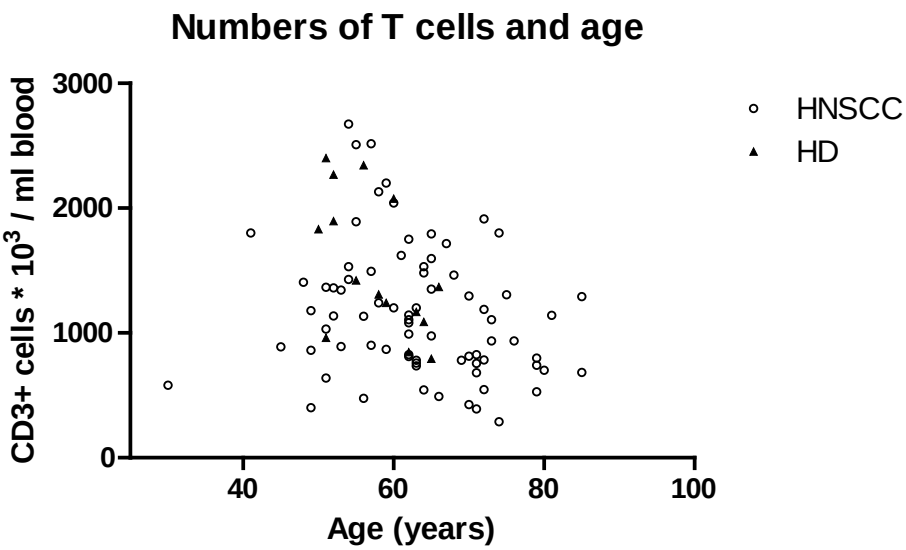

Figure 1 Absolute numbers of T cells decrease with age. *The absolute number of $CD3^+$ cells measured in peripheral blood per millilitre was plotted against age in years. Patients with HNSCC (open circles, range 30–86 years), HD (closed triangles, range 50–66 years)*

Differences in peripheral T cell subpopulations between HD and HNSCC patients

Next we analysed the relative numbers of naive, central memory, effector memory and effector T cells. Based on literature (Hamann *et al*, 1997; Di *et al*, 2011), naive T cells were defined as $CD27^+$ $CD45RA^+CD45RO^-$; central memory as $CD27^+$ $CD45RO^+$ $CD45RA^-$; effector memory as $CD27^-CD45RO^+CD45RA^-$; and effector T cells as $CD27^-CD45RO^-$ $CD45RA^+$. Figure 3 shows these T cell subpopulations for HD and all patients with HNSCC together as a percentage of total $CD3^+$ cells. From these data, it is clear that patients with HNSCC had a significantly lower percentage of naive T cells (Figure 3a; $P < 0.01$) and higher percentages of central memory T cells (Figure 3b; $P < 0.05$) and effector memory T cells (Figure 3c; $P < 0.005$), as compared to HD. The percentage of effector T cells (Figure 3d) did not differ between patients with HNSCC and HD. A correlation between age and T cell subpopulation distribution was not found.

Tumour stage and differences in peripheral T cell subpopulations

Tumour stage is an important prognostic factor for patients with HNSCC. In Figure 4, the percentages of the four different T cell subpopulations described above are shown for stages I, II, III or IV patients with HNSCC and for HD. The percentage of naive T cells was significantly lower in stage II patients with HNSCC compared with HD ($P < 0.01$; Figure 4a). No significant differences between HD and HNSCC patients in the four different stages were found for the central memory T cells (Figure 4b). Patients with stages II and III tumours had more effector memory T cells than HD ($P < 0.01$ and $P < 0.005$ respectively; Figure 4c). No significant differences between HD and HNSCC patients in the four different stages were found in the effector T cell population (Figure 4d). The percentage of naive, central memory, effector memory and effector T cells did not differ

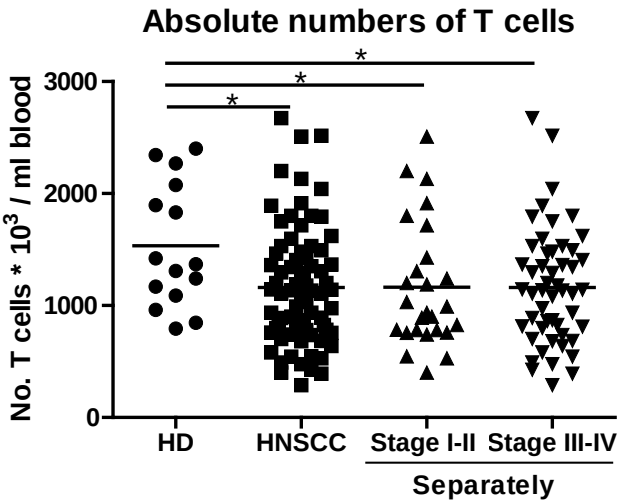

Figure 2 Patients with HNSCC have lower numbers of circulating T cells than healthy donors. *Absolute numbers of lymphocytes per millilitre peripheral blood are shown for HD, the whole group of Patients with HNSCC and patients with HNSCC grouped in early stage (stage I/II) and advanced stage (stage III/IV) patients (*, $P < 0.05$)*

significantly between tumour stages (Figure 4a–d). Furthermore, the percentage of $CD56^+$ T cells was assessed as CD56 expression has been associated with T cell effector function (cytolytic activity as well as cytokine production) (Kelly-Rogers *et al*, 2006; Pievani *et al*, 2011). Figure 4e shows the percentages of $CD56^+$ T cells for HD and HNSCC patients. The percentages of $CD56^+$ T cells followed the same trend in tumour stage distribution as the effector memory T cells (Figure 4c,e), a higher percentage in stages II and III compared with stages I and IV. Figure 4F shows the correlation between $CD56^+$ T cells and effector memory T cells of circulating $CD3^+$ cells in the patients with HNSCC. High percentages of $CD56^+$ T cells correlated with high percentages of effector memory T cells (Figure 4f) and of effector T cells (Supplementary Figure S1a). In keeping with this, low percentages of $CD56^+$ T cells correlated with high percentages of naive T cells (Supplementary Figure S1b).

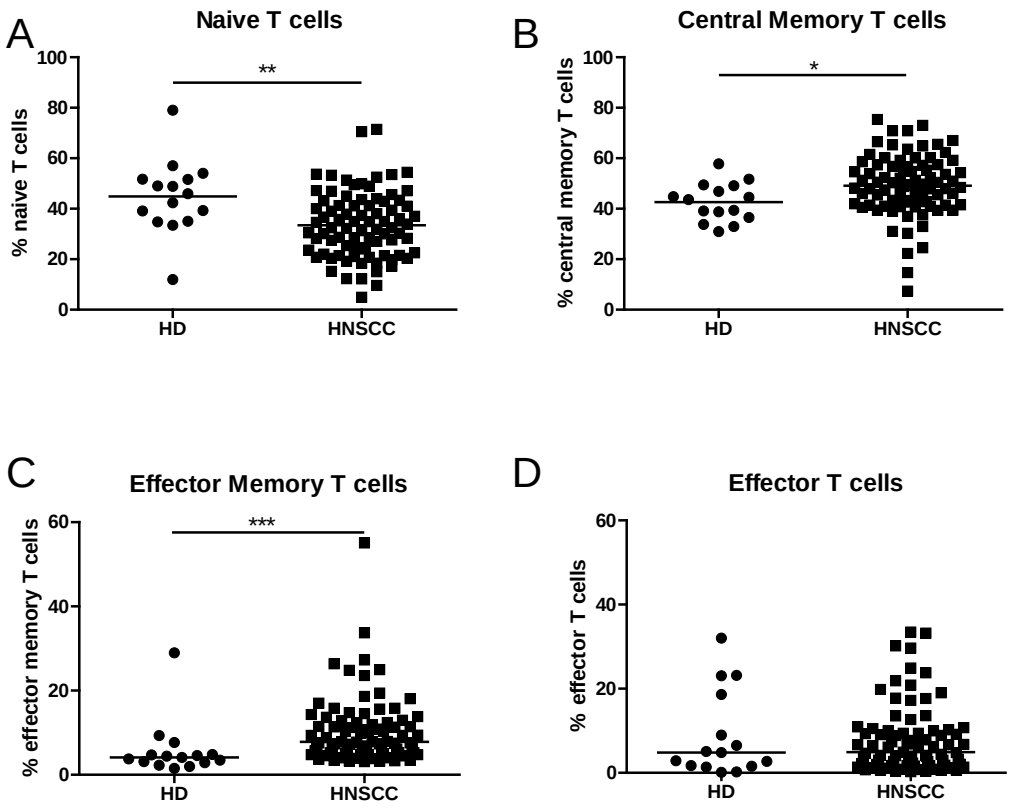

Figure 3 T cell subpopulations in patients with HNSCC and healthy donors. *$CD3^+$ T subpopulations are shown for HD vs HNSCC patients:* ***(a)*** *Naive T cells $CD3^+/CD27^+/CD45RA^+$,* ***(b)*** *Central memory T cells $CD3^+/CD27^+/CD45RO^+$,* ***(c)*** *Effector memory T cells $CD3^+/CD27^-/CD45RO^+$ and* ***(d)*** *Effector T cells $CD3^+/CD27^-/CD45RA^+$ (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$)*

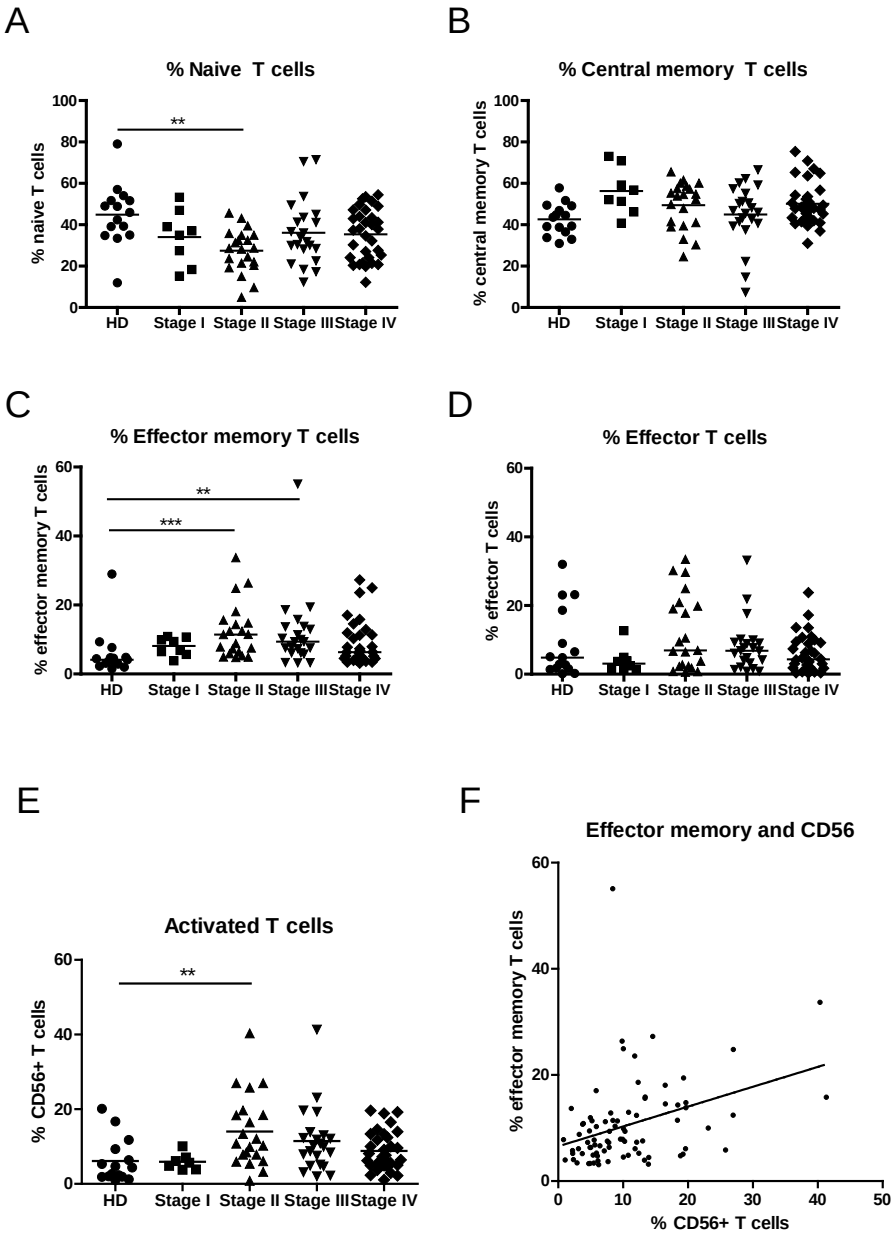

Figure 4 T cell subpopulations and tumour stage. *Shown are T cell in relation to tumour stage at presentation in patients with HNSSC compared with HD. Horizontal lines represent means.* ***(a)*** *Naive T cells $CD3^+/CD27^+/$ CD45RA (ANOVA, Tukey's post-test **, $P < 0.01$).* ***(b)*** *Central memory T cells $CD3^+/$ $CD27^+/CD45RO^+$.* ***(c)*** *Effector memory T cells $CD3^+/CD27^-/$ $CD45RO^+$, (Kruskal–Wallis test with Dunn's post-test ***, $P < 0.005$ and **, $P < 0.01$).* ***(d)*** *Effector T cells $CD3^+/CD27^-/$ $CD45RA^+$.* ***(e)*** *Activated T cells $CD56^+$, (Kruskal–Wallis test with Dunn's post-test **, $P < 0.01$).* ***(f)*** *The percentage of effector memory T cells plotted against the percentage of activated ($CD56^+$) T cells for all patients with HNSCC ($R = 0.37$, $P < 0.001$)*

Tumour site and peripheral T cell phenotype

We also investigated the distribution of T cell subpopulations in all patients with HNSCC in relation to the site of the primary tumour (oral cavity, oropharynx, hypopharynx and larynx). The percentage of naive T cells was significantly lower in patients with larynx ($P < 0.05$) or oropharynx ($P < 0.05$) cancers compared with HD (Figure 5a). No significant differences were found in the percentages of central memory T cells between HD and HNSCC patients grouped per site of origin (Figure 5b). We observed an increase in effector memory T cells in the patients with tumours arising from the oral cavity ($P < 0.01$), oropharynx ($P < 0.01$) and larynx ($P < 0.01$; Figure 5c). No significant differences were found in the percentages of effector T cells between HD and HNSCC patients grouped per site of origin (Figure 5d). We found no differences in T cell subpopulations between the patients grouped per tumour site (Figure 5a–d).

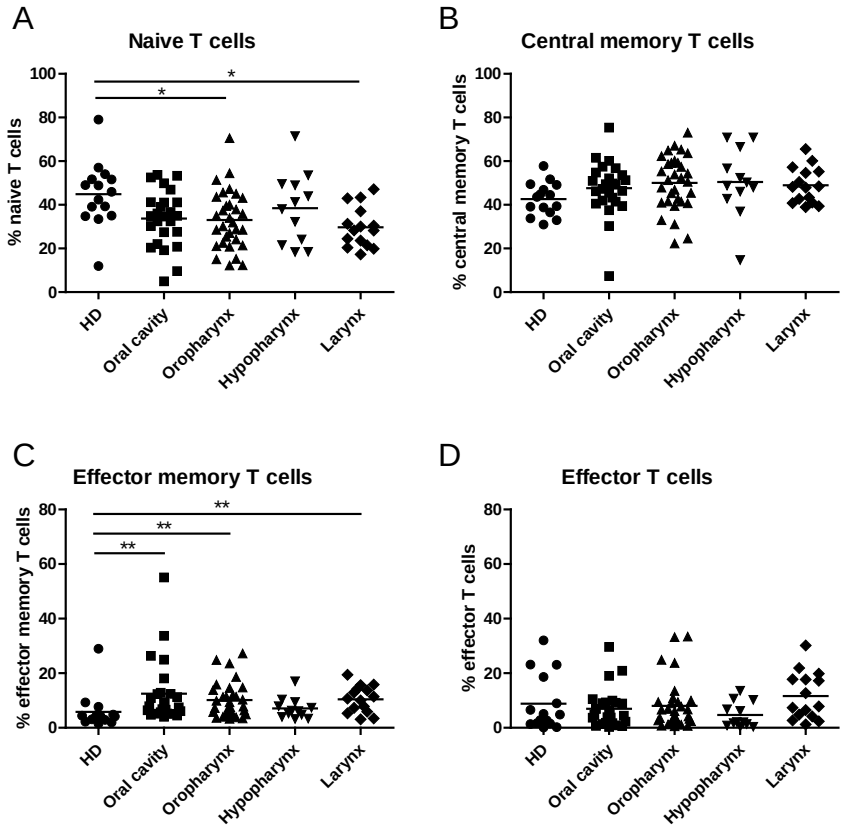

Figure 5 T cell subpopulations and site of origin. *Shown are T cell in relation to tumour stage at presentation in patients with HNSSC compared with HD. Horizontal lines represent means.* ***(a)*** *Naive T cells CD3⁺/CD27⁺/ CD45RA (ANOVA, Tukey's post-test *, $P < 0.05$).* ***(b)*** *Central memory T cells CD3⁺/ CD27⁺/CD45RO⁺.* ***(c)*** *Effector memory T cells CD3⁺/CD27⁻/ CD45RO⁺, (Kruskal–Wallis test with Dunn's post-test **, $P < 0.01$).* ***(d)*** *Effector T cells CD3⁺/ CD27⁻/CD45RA⁺*

HPV status of OPSCC and T cell phenotype

Next we analysed T cell subpopulations in patients with HPV-positive (N = 9) vs HPV-negative (N = 16) oropharynx squamous cell carcinoma (OPSCC). No significant differences were found in the absolute number of circulating $CD3^+$ T cells between patients with HPV-positive or HPV- negative OPSCC [mean: 1004 and 1185; median: 1120 and 1140 ($*10^3$) per mL of blood, respectively]. Figure 6 shows a comparison between the T cell subsets in patients with HPV-positive vs HPV-negative OPSCC. Although the number of cases per subgroup was relatively low, the percentage of naive T cells was significantly lower in the patient group with HPV-positive OPSCC compared with the patient group with HPV-negative OPSCC ($P < 0.05$; Figure 6a). The percentage of central memory T cells in oropharyngeal tumour patients did not differ between HPV-positive and HPV-negative cases (Figure 6b). The percentages of effector memory T cells were significantly higher in patients with HPV-positive OPSCC compared with patients with HPV-negative OPSCC ($P < 0.05$; Figure 6c), as were the percentages of effector T cells ($P < 0.05$; Figure 6d). Thus, patients with HPV-positive OPSCC had significantly lower percentages of naive T cells and higher percentages of effector (memory) T cells compared with patients with HPV-negative OPSCC.

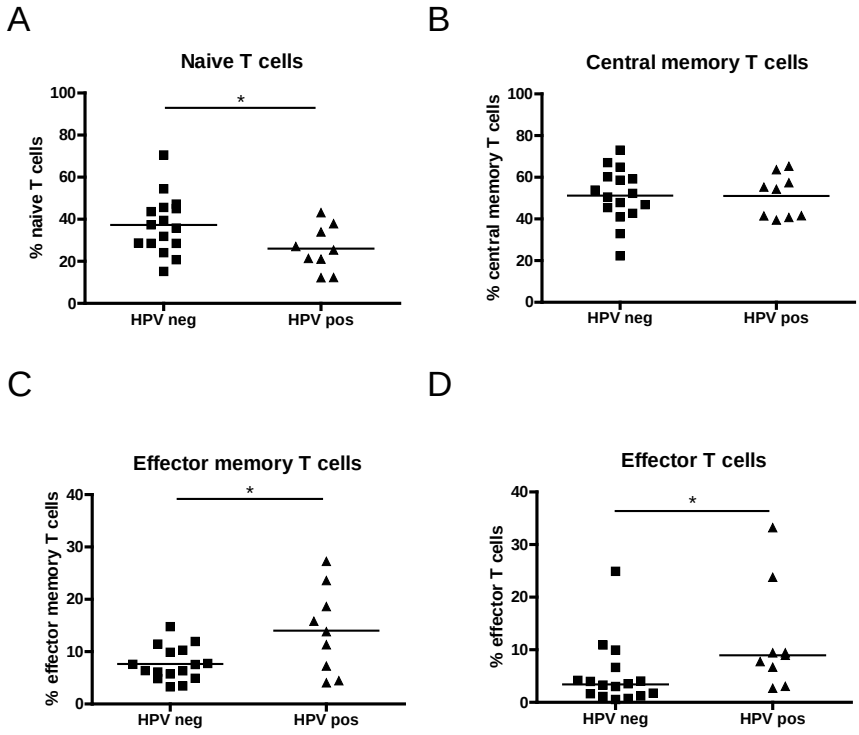

Figure 6 T cell subpopulations and HPV status. *CD3+ T cell subpopulations percentages in oropharyngeal squamous cell carcinoma patients divided according to HPV status. Horizontal lines represent means.* ***(a)*** *Naive T cells CD3+/CD27+/CD45RA+ (Student's t-test, * $P < 0.05$).* ***(b)*** *Central memory T cells CD3+/ CD27+/CD45RO+.* ***(c)*** *Effector memory T cells CD3+/CD27-/CD45RO+ (Student's t-test * $P < 0.05$).* ***(d)*** *Effector T cells CD3+/CD27-/CD45RA+ (Mann–Whitney U-test * $P < 0.05$)*

Discussion

To design effective immune therapy for patients with HNSCC, knowledge about the status of the immune systems of these patients is important. Immunocompetent patients are more likely to benefit from active immunization strategies such as dendritic cell vaccination (Yang *et al*, 2010). In cases of an immune compromised state, patients will more likely benefit from passive immunotherapy such as adoptive T cell transfer (Jorritsma *et al*, 2009; Scholten *et al*, 2011). Data about the immune status of a patient could be predictive of outcome even for conventional therapies like chemotherapy (Zitvogel *et al*, 2011).

As expected, the number of circulating T cells decreased with age in the current patient population, which is in agreement with published data (Kuss *et al*, 2004; Linton and Dorshkind, 2004; Woodland and Blackman, 2006). We avoided a potential negative effect of treatment on the number and distribution of T cell subsets by analysing the immunophenotypes at the time of diagnosis, so prior to treatment. Four-colour FACS analysis was performed on full blood samples circumventing extensive sample handling like density gradient separations or cell sorting. In doing so, only a small volume of blood is needed for the analyses. The down side of this procedure is that the presence of scarce T cell subsets like regulatory T cells and tumour-specific T cells cannot be charted. For example, in patients with HPV-positive tumours, it would be interesting to chart the presence of HPV-specific T cells. In the past, we have used MHC class I tetramers to isolate HPV-specific T cells after repetitive *in vitro* stimulation (Schreurs *et al*, 2003). These $CD8^+$ T cells are however of lesser clinical importance. Clinical benefit correlated with CD4-mediated HPV-specific T cell responses (Steele *et al*, 2005; Melief and van der Burg, 2008; Stanley, 2009). Visualization of tumour-specific T cells by means of tetramer staining would be feasible for a limited number of antigenic peptide/MHC class I or class II combinations only, and necessitating examination of large volumes of peripheral blood.

The current pilot study was designed to easily chart numbers and percentages of T cell subsets present in the blood of patients with HNSCC. The data obtained will be correlated with clinical outcome when recurrence free and overall survival data will be available.

The various T cell subpopulations were not significantly different, between the different tumour stages. It was noted, however, that the percentage of effector memory T cells increased from HD to stages I and II tumour patients and subsequently declined again in stages III and IV tumour patients. The reverse trend was noted for naive T cells. It can be envisioned that small (stage I) tumours have limited effects on the immune system of patients with HNSCC, which may increase with tumour load in stage II (tumour-specific T cell priming: hence a shift from naive to memory subpopulations). At a later stage, tumours may exert immune suppressive effects, leading to a decline in effector (memory) T cells.

Although no significant differences were seen between different primary tumour sites, we did observe that patients with tumours originating from the oropharynx had more effector memory T cells. This may be expected because OPSCC can arise from tonsils that consist of lymphoid tissue perhaps being capable of a more efficient immune response than tumours arising from other sites. We investigated the differences in T cell subsets between patients with HPV-positive and HPV-negative OPSCC. In the patients with an HPV-positive status, higher percentages of effector (memory) T cells were observed compared with patients with an HPV-negative status. These findings could be an indication for the presence

of anti-HPV immunity as has been described recently by Heusinkveld *et al* (Heusinkveld *et al*, 2012). HPV-specific immunity may contribute to the improved survival observed for patients with HPV-positive OPSCC.

It has been documented that activated CD4-positive tumour infiltrating lymphocytes (TIL) correlate with a better prognosis, indicating that the activation level of T cells in patients with HNSCC is important for survival (Badoual *et al*, 2006). Wanson *et al* observed that numbers of TIL correlated with disease-specific survival. A good correlation was also found between disease-specific survival and HPV status. However, they did not find significantly more TIL in HPV-positive patients (Wansom *et al*, 2012). This suggests that an ongoing immune response plays an important role in survival of patients with HNSCC, regardless of HPV status. Here, we documented that patients with HPV-positive SCC have high percentages of effector memory T cells. In the patient population with HPV-negative SCC, we also found a number of patients with high percentages of effector memory T cells. In follow-up studies, we will investigate whether high percentages of effector memory T cells correlated to a favourable prognosis, independent of HPV status. The relatively small number of HD controls is a noted limitation of the current study. Studies with larger numbers of HD and patient samples are warranted to confirm our findings.

In conclusion, increased effector memory T cell rates in patients with HPV-positive oropharyngeal squamous cell carcinomas suggest a higher level of intrinsic immunogenicity of these virus-associated tumours. Similarly, patients with stage II tumours displayed a systemic shift from naive to memory T cells. This inventory of T lymphocytes in the peripheral blood of patients with HNSCC may provide a simple and easily implemented tool for prognosis and/or therapy response prediction.

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Supporting Information

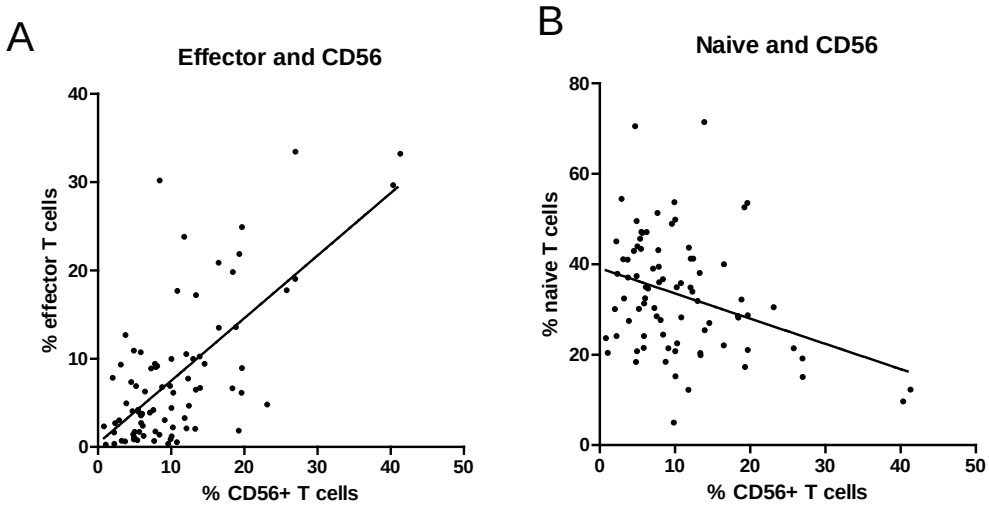

Figure S1 Effector en naive T cells correlated to activated T cells. (a) *The percentage of effector T cells plotted against the percentage of activated ($CD56^+$) T cells for all patients with HNSCC ($R = 0.47$, $P < 0.0001$).* ***(b)*** *The percentage of naive T cells plotted against the percentage of activated ($CD56^+$) T cells for all patients with HNSCC ($R = 0.11$, $P < 0.0022$).*