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# Liver transplant recipients have a higher prevalence of anal squamous intraepithelial lesions

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**Background:** Anal squamous intraepithelial lesions (ASIL) are precancerous lesions of anal squamous cell carcinoma, with a higher prevalence in immunosuppressed patients. There are some studies in kidney transplant recipients, but there is no information regarding prevalence in liver transplantation. Our aim was to evaluate the prevalence of ASIL in this setting.

**Methods:** Prospective case–control study involving liver transplant recipients without any other known risk factor for ASIL ( $n = 59$ ), which were compared with a healthy control group ( $n = 57$ ). All were submitted to anal cytology and high-resolution anoscopy was performed in those with abnormal results.

**Results:** Ten (17%) of liver transplant recipients had abnormal cytological results, seven patients had atypical squamous cells of undetermined significance (ASC-US), one patient had atypical squamous cells that cannot exclude high-grade (ASC-H) and two patients had high-grade squamous intraepithelial lesions (HSIL). In the control group, one patient (2%) had an ASC-US result ( $P = 0.005$ ). Anal squamous intraepithelial lesions were confirmed in 7 out of 10 of liver transplant patients and 0 out of 1 in the controls ( $P = 0.013$ ) by high-resolution anoscopy with biopsies. Current smoking was the only risk factor for abnormal cytology (odds ratio = 5.87, 95% confidence intervals = 1.22–28.12,  $P = 0.027$ ).

**Conclusions:** Liver transplant patients have a higher risk of ASIL. Screening should be considered, especially in smokers.

Anal squamous intraepithelial lesions (ASIL) or anal intraepithelial neoplasia are precancerous lesions of anal squamous cell carcinoma (SCC) and are related to human papillomavirus (HPV) infection (Palefsky *et al*, 1991; Frisch *et al*, 1997; Varnai *et al*, 2006). Recently published cancer incidence and mortality

projections showed that anal cancer will be one of the fastest growing cancers in terms of incidence and mortality in the United Kingdom over the next two decades (Smittenaar *et al*, 2016).

Cervical carcinogenesis and anal carcinogenesis have several important similarities, namely the role of HPV, anatomic origin

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(squamocolumnar junction) and the same precancerous lesions (Roberts and Thurloe, 2012). In cervical screening, women are normally tested by cervical cytology and those with more suspicious results are then referred for colposcopy. If high-grade squamous intraepithelial lesions (HSIL) are confirmed, the patient is normally treated, in an effort to prevent progression to cervical cancer (Massad *et al*, 2013). The rationale for anal cancer screening follows the same principles as cervical cancer screening and focusses in high-risk groups (Roberts and Thurloe, 2012; Hillman *et al*, 2016). There are some groups that have been recognised as higher risk for ASIL and anal SCC, namely men who have sex with men (MSM), human immunodeficiency virus (HIV)-infected individuals (Machalek *et al*, 2012) and women with a history of lower genital tract neoplasia (Moscicki *et al*, 2015). Kidney transplant recipients also have a higher prevalence of ASIL (Ogunbiyi *et al*, 1994) and the anal cancer relative risk is 10-fold higher than in non-immunosuppressed patients (Adami *et al*, 2003; Patel *et al*, 2010). Previous studies showed a 20% prevalence of ASIL in kidney transplant recipients and a 47% anal infection rate of HPV 16 (Ogunbiyi *et al*, 1994).

Although some information exists in liver transplantation concerning HPV infection, there are no studies determining the prevalence of ASIL in this population. Our aim was to evaluate the prevalence of ASIL in liver transplant recipients compared with healthy subjects.

## MATERIAL AND METHODS

**Study design, inclusion and exclusion criteria.** This was a prospective case-control study involving liver transplant recipients that were compared with a healthy control group. Liver transplant recipients were followed at the Liver and Pancreatic Transplant Unit at Centro Hospitalar Porto and in the Gastroenterology Department of Centro Hospitalar S. João. Only liver transplant recipients older than or equal to 18 years and transplanted for  $\geq 2$  years were included. The sex-matched healthy control group included adult patients referred to colonoscopy or that were being followed in the Gastroenterology outpatient clinic of the Gastroenterology Department of Centro Hospitalar S. João.

Patients transplanted due to familial amyloid neuropathies were not included. MSM, HIV positive, women with a history of lower genital tract neoplasia, HPV vaccination and previous history of ASIL or anal cancer patients were excluded. Patients under immunosuppressive drugs for other causes, besides liver transplantation, inflammatory bowel disease (IBD), anorectal pathology (fissure, fistula, abscess, large hemorrhoids), sexually transmitted diseases or previous cancer submitted to chemotherapy or radiotherapy, were also excluded. A history of anal receptive intercourse was considered an exclusion criteria for transplanted or control men, but not for women. No men in both groups reported practising receptive anal intercourse before study entrance. One male patient of the control group that had an abnormal cytology result confirmed, after the result was obtained, that he was a MSM. Another control subject had an anal cytology that showed the presence of cells with suspicious eosinophilic nuclear inclusions, suggesting a Herpes infection (no sign of infection was present during anal cytology collection) and he then refused further evaluation. Both patients were excluded from the final analysis (MSM and/or DST were exclusion criteria) that only included 57 controls and not the initial 59 subjects.

The study was approved by the Health Ethics Committee of Centro Hospitalar S. João (reference CES 59-16) and Centro Hospitalar Porto (reference 2016.145 124-DEFI/117-CES), and was performed in accordance with the 1964 Declaration of Helsinki and its later amendments.

**Study visit and data collection.** Random transplant recipients fulfilling the study criteria were enrolled during a routine outpatient visit. The study was explained and the informed verbal and written consent obtained from all patients that accepted entering the study. Information on age, aetiology of liver disease, liver transplantation date, type and duration of immunosuppression, HIV infection, cancer, lower anogenital tract neoplasia or IBD diagnosis, smoking, HPV vaccination, sexual orientation, marital status, age at first sexual intercourse, number ( $\leq$  or  $> 3$ ) lifetime sexual partners, history of receptive anal intercourse and last defecation time before anal cytology ( $\leq$  or  $> 1$  h) were recorded. After the interview, anal cytology was collected.

After full explanation of the study and the required procedures, two liver transplant recipients refused participation. None of the healthy controls refused participation.

**Procedures: anal cytology and high-resolution anoscopy.** All patients were submitted to anal cytology. Those with abnormal cytological results, namely HSIL, atypical squamous cells that cannot exclude high-grade squamous intraepithelial lesions (ASC-H), low-grade squamous intraepithelial lesions (LSIL) and atypical squamous cells of undetermined significance (ASC-US), were further evaluated with high-resolution anoscopy (HRA) and biopsies of any high-grade suspicious lesion. Only patients with a negative result for intraepithelial lesion or malignancy (NILM) were not referred.

Anal cytology was performed using a sterile polyester swab (Thermo Fisher Scientific, Waltham, MA, USA), previously moistened with water with the patients in the left lateral decubitus position. The swab was inserted in the distal rectum and then slowly withdrawn with rotating movements during 20 s. Samples were placed into PreservCyt ThinPrep solution (Hologic UK, Crawley, UK). Four experienced clinicians collected the anal cytologies: AA, SR, RG, RM; the last three were previously trained by AA.

High-resolution anoscopy was performed using a Carl Zeiss colposcope (Carl Zeiss, Oberkochen, Germany) and all procedures were performed by AA, with more than 3 years of experience and a mean number of 400 procedures per year in HRA. Patients were observed in the knee-chest position without any previous bowel preparation. A disposable anoscope was inserted and the colposcope was used to examine the squamocolumnar junction, the anal canal and the perianal skin, initially without any staining and then with the topical application of 5% acetic acid and Lugol's iodine solution (anal application exclusively). When high-grade disease was suspected, anal and/or perianal lesions were submitted to biopsies using a mini-Tischler punch-biopsy forceps. No previous local anaesthesia was normally necessary for anal biopsies and 1% lidocaine buffered with 8.4% of sodium bicarbonate was used for perianal biopsies.

**Sample processing.** All anal cytologies and biopsies were analysed in the Pathology Department of Centro Hospitalar S. João in Porto. Two cytopathologists analysed the samples blinded to the subject group and when discordant results were obtained a third opinion was recorded. Classification of cytology was done according to the Bethesda System in HSIL, ASC-H, LSIL, ASC-US and NILM. Histology was classified in LSIL and HSILs according to the Lower Anogenital Squamous Terminology (Darragh *et al*, 2012).

**Statistic analysis.** Continuous variables were described as mean  $\pm$  standard deviation or median (interquartile range) according to the distribution symmetry. Categorical variables were described as absolute and relative frequencies. The comparison between groups was made using the Student's *t*-test for parametric or the Mann-Whitney *U*-test for non-parametric continuous variable and the  $\chi^2$ -test or Fisher's exact test for categorical variables. To determine factors that were associated with cytological alterations,

univariate and adjusted odds ratios (ORs) with a 95% confidence intervals (CIs) were calculated using logistic regression. Two models of multivariate logistic regression were also developed using a limited number of variables due to the number of cases. One model included current smoking, age and hepatitis B and the other current smoking, age and time after transplantation. A significance level of  $\alpha = 5\%$  was considered in all hypothesis tests.

For current smokers, the number of pack-years was calculated as the number of cigarettes smoked per day/20  $\times$  number of years smoked.

For the sample size calculation, an expected ASIL prevalence of 20% in the liver transplant group and 2% in controls was used, with a ratio of controls to cases 1:1, an 80% power and 95% confidence. Previous studies have showed an ASIL prevalence of 20% of the kidney transplant population (Ogunbiyi *et al*, 1994) and 0.7% to 2.6% in healthy controls (Ogunbiyi *et al*, 1994; Jacyntho *et al*, 2011). A sample of at least 57 patients in each group was deemed necessary.

Statistic analysis was performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

## RESULTS

A total of 59 liver transplant recipients and 57 controls were included. In the liver transplant group, 37 patients (63%) were men, with a mean age of  $54 \pm 10$  years. In the healthy control group, 36 subjects (63%) were men, with a mean age  $59 \pm 11$  years. There were no statistically significant differences between groups concerning sex or smoking habits, but liver transplant recipients were younger (Table 1).

The most common aetiology for liver transplantation was alcoholic in 26 patients (44%), hepatitis B in 7 patients (12%), and hepatitis C and autoimmune hepatitis each in 6 patients (10%). The most commonly used drug therapy was tacrolimus only ( $n = 23$ , 39%) or tacrolimus and mycophenolate mofetil ( $n = 10$ , 17%). Seven patients were also on prednisolone (12%), associated with other drugs. Regarding immunosuppression duration, the median was 7 (5–11) years. Nine patients were current smokers (15%), 8 in 22 women had previous anal intercourse (36%) and 47 patients were married (80%).

Regarding anal cytology, 10 of liver recipients (17%) had abnormal results, 7 patients had ASC-US, 1 patient ASC-H and 2 patients HSIL. In the control group, one subject (2%) had abnormal cytology with ASC-US ( $P = 0.005$ ) (Table 1). All anal cytology samples were considered satisfactory for analysis and 87 out of 116 (75%) were representative of the transformation zone (no statistically significant difference between the two groups).

In the univariate logistic regression, current smoking was the only risk factor for abnormal cytological results (OR = 5.87, 95% CI = 1.22–28.12,  $P = 0.027$ ) (Table 2). Liver transplant recipients with abnormal anal cytological results had a higher number of pack-years smoked ( $P = 0.012$ ) with a median of 18 (15–35) than patients with normal cytological results with a median of 10 (4–10). Ever smoking (OR = 1.85, 95% CI = 0.45–7.60,  $P = 0.396$ ) was not a risk factor for cytological abnormalities. Regarding immunosuppression, current therapy, current doses and the duration of immunosuppression (time after transplantation) were also not risk factors for abnormal results in liver transplant recipients.

Two multivariate models were developed using current smoking, as the only statistically significant factor in the univariate analysis (although current smoking was the only factor, different adjustments were done using possibly clinically significant variables). One model included current smoking, age and hepatitis B and the other current smoking, age and time after

**Table 1. Comparison between liver transplant patients ( $n = 59$ ) and the control group ( $n = 57$ )**

Parameter	Control group	Liver transplant	P-value
Men, n (%)	36 (63)	37 (63)	0.960 <sup>a</sup>
Age (years), mean $\pm$ s.d.	$59 \pm 11$	$54 \pm 10$	0.026 <sup>b</sup>
Current smoking, n (%)	10 (18)	9 (15)	0.739 <sup>a</sup>
Abnormal anal cytology, n (%)	1 (2)	10 (17)	0.005 <sup>a</sup>
NILM, n (%)	56 (98)	49 (83)	—
ASC-US, n (%)	1 (2)	7 (12)	
LSIL, n (%)	0 (0)	0 (0)	
ASC-H, n (%)	0 (0)	1 (2)	
HSIL, n (%)	0 (0)	2 (3)	
Satisfactory cytology	57 (100)	59 (100)	—
Histologic abnormalities, n (%)	0 (0)	7 (12)	0.013 <sup>c</sup>
Negative/normal, n (%)	1 (100)	3 (30)	—
LSIL, n (%)	0 (0)	5 (50)	
HSIL, n (%)	0 (0)	2 (20)	

Abbreviations: ASC-H=atypical squamous cells which cannot exclude high-grade squamous intraepithelial lesions; ASC-US=atypical squamous cells of undetermined significance; HSIL=high-grade squamous intraepithelial lesions; LSIL=low-grade squamous intraepithelial lesions; NILM=negative for intraepithelial lesion or malignancy.

<sup>a</sup> $\chi^2$ -test.  
<sup>b</sup>Student's t-test.  
<sup>c</sup>Fisher's exact test.

transplantation. Current smoking remained the only risk factor for cytological abnormalities (Table 3).

High-resolution anoscopy was performed in all patients with abnormal cytology results. Anal squamous intraepithelial lesions were histologically confirmed in 7 out of 10 of liver transplant patients and 0 out of 1 in the control group ( $P = 0.013$ ). In total, five liver transplant patients had LSIL and two patients had HSIL (Table 1). Correspondence between abnormal cytology and histological results for liver transplant recipients can be seen in Table 4.

## DISCUSSION

There are no previous published data on ASIL prevalence in liver transplant recipients. Information in the transplant setting is limited to very few studies, exclusively in kidney transplant patients. Ogunbiyi *et al* (1994) performed a prospective case control study including 133 kidney transplant recipients and 145 controls submitted to HRA and HPV testing. Anal squamous intraepithelial lesion was present in 26 out of 133 (20%, with 5% HSIL) of transplant recipients vs 1 out of 145 (0.7%) of controls. All patients with anal HSIL, except one, had previous or concurrent lower genital tract neoplasia and these patients were not excluded from the analysis. Ogilvie *et al* (2008) conducted a study involving 40 kidney transplant recipients (for more than 6 months) submitted to anal cytology, HPV and HRA. No control group was used. Results showed that abnormal anal cytology was present in 2/35 patients (6%) and HRA with histology confirmed ASIL in 6 out of 39 patients (15%) with 5% of HSIL. Patel *et al* (2010) conducted a study including 108 kidney transplant patients tested with anal cytology and abnormal results were detected in 10 out of 104 (9.6%). Results of the following anoscopy/HRA/biopsies were not provided and no control group was used. Besides the lack of publications, previous data on kidney transplantation have important limitations (inclusion of patients with other risk factors for ASIL, lack of a control group or absence of following up abnormal cytology results with HRA) that make more robust conclusions difficult to draw. This study was designed to overcome previous limitations.

**Table 2. Comparison between liver transplant patients with (n = 49) and without anal cytology abnormalities (n = 10) and univariate logistic regression analysis**

Parameter	Normal cytology		Abnormal cytology		P-value <sup>a</sup>	Univariate logistic regression		
	n	%	n	%		OR	IC 95%	P-value
<b>Sex</b>								
Men	31	63	6	60	> 0.999	1.15	Ref. 0.29–4.62	0.846
Women	18	37	4	40				
<b>Age (years)</b>								
< 50	13	27	4	40	0.453	0.54	Ref. 0.13–2.23	0.396
≥ 50	36	73	6	60				
<b>Aetiology</b>								
Alcohol	21	43	5	50	0.736	1.33	0.34–5.21	0.679
Hepatitis B	5	10	2	20	0.590	2.20	0.36–13.37	0.392
Hepatitis C	6	12	0	0	0.365	—	—	—
Autoimmune Hepatitis	5	10	1	10	> 0.999	0.98	0.10–9.40	0.984
<b>Current drugs</b>								
Tacrolimus	33	67	6	60	0.721	0.73	0.18–2.95	0.655
Mycophenolate mofetil	22	45	3	30	0.221	0.53	0.12–2.28	0.390
Cyclosporine	11	22	4	40	0.257	2.30	0.55–9.64	0.254
Sirolimus	4	8	0	0	0.660	—	—	—
Everolimus	2	4	1	10	0.320	2.61	0.21–31.94	0.453
Prednisolone	6	12	1	10	> 0.999	0.80	0.09–7.45	0.842
Azathioprine	1	2	0	0	> 0.999	—	—	—
Current smoking	5	10	4	40	0.008	5.87	1.22–28.12	0.027
<b>Sexual partners lifetime</b>								
≤ 3 Partners	27	55	5	50	0.692	1.23	Ref. 0.32–4.79	0.768
> 3 Partners	22	45	5	50				
Anal intercourse	7	39	1	25	> 0.999	0.52	0.05–6.09	0.605
<b>Marital status</b>								
Single	4	8	1	10	0.888	1.03	0.19–5.60	0.977
Married	39	80	8	80				
Divorced	4	8	1	10				
Widow	2	4	0	0				
<b>First sexual intercourse</b>								
< 18 Years	24	49	8	80	0.092	0.24	Ref. 0.05–1.25	0.090
≥ 18 Years	25	51	2	20				
<b>Time of last defecation</b>								
≤ 1 h	7	14	0	0	0.590	—	—	—
> 1 h	42	86	10	100				
<b>Time after transplantation</b>								
≥ 2 And < 5 years	9	18	3	30	0.746	0.48	Ref. 0.09–2.58	0.392
≥ 5 And < 10 years	25	51	4	40				
≥ 10 Years	15	31	3	30				

Abbreviations: IC 95 = 95% confidence interval; OR = odds ratio.  
<sup>a</sup>χ<sup>2</sup>-test or Fisher's exact test.

**Table 3. Multivariate logistic regression analysis of cytological abnormalities**

	Adjusted OR	IC 95%	P-value
<b>Model 1</b>			
Current smoking	5.55	1.07–28.72	0.041
Hepatitis B	1.17	0.16–8.81	0.877
Age	0.98	0.91–1.06	0.587
<b>Model 2</b>			
Current smoking	7.41	1.34–40.98	0.022
Time after transplantation			
≥ 2 And < 5 years	Ref.		
≥ 5 And < 10 years	0.32	0.05–2.11	0.234
≥ 10 Years	0.49	0.07–3.46	0.474
Age	0.99	0.91–1.07	0.727

Abbreviations: IC 95 = 95% confidence interval; OR = odds ratio.

In our analysis, a 17% prevalence of abnormal cytology was obtained in liver transplant recipients (n = 10), significantly higher than in controls (P = 0.005). Histologic confirmation of ASIL was obtained in seven patients, two with HSIL. Patients and controls that might have had other possible ASIL risks factors (other than post-transplant immunosuppression) were not included. Familial amyloid neuropathy is a common cause of liver transplantation in the Portuguese population and to select a representative and homogeneous liver transplant group, these patients were not included. Only patients with over a 2-year history of transplantation were included because of the more stable immunosuppression and the possible necessary time for anal HPV-related lesions to appear, although ASIL natural history, time and rate of progression is largely unknown. In kidney transplantation, anal lesions seem to appear < 5 years after transplantation and increase significantly up to 10 years, when it levels out (Ogunbiyi *et al*, 1994), but results are conflicting (Ogilvie *et al*, 2008). In a previous study in HPV-related cancers after solid organ transplantation (Madeleine *et al*, 2013),

**Table 4. Description of the liver transplant recipients with abnormal cytology (n = 10)**

Age	Sex	Aetiology	Current drugs	Current smoker	Years of transplant	Cytology	Histology	Location of lesions	Number of lesions
72	Men	Alcohol	MMF + CYC	No	8	ASC-US	NEGATIVE	—	—
56	Men	Alcohol	TAC	No	2	ASC-US	NEGATIVE	—	—
54	Women	PBC	MMF + CYC	No	3	ASC-US	NEGATIVE	—	—
45	Men	Alcohol	TAC	Yes	4	ASC-US	LSIL	Anal	1
51	Men	Autoimmune hepatitis	TAC	Yes	5	ASC-US	LSIL	Anal	2
56	Women	Alcohol	TAC	No	11	ASC-US	LSIL	Anal	2
65	Women	Alcohol	EVE + CYC	No	7	ASC-US	LSIL	Anal + perianal	4
49	Men	Hepatitis B	CYC	Yes	16	ASC-H	LSIL	Anal	1
33	Women	Wilson	TAC + MMF + PDN	No	5	HSIL	HSIL	Anal	1
44	Men	Hepatitis B	TAC	Yes	11	HSIL	HSIL	Anal	2

Abbreviations: ASC-H = atypical squamous cells which cannot exclude high-grade squamous intraepithelial lesions; ASC-US = atypical squamous cells of undetermined significance; CYC = cyclosporine; EVE = everolimus; HSIL = high-grade squamous intraepithelial lesions; LSIL = low-grade squamous intraepithelial lesions; MMF = mycophenolate mofetil; PBC = primary biliary cholangitis; PDN = prednisolone; TAC = tacrolimus.

the time from transplant to diagnosis of *in situ* (HSIL) and invasive cancers (including anal cancers) ranged from a median of 2.6–5.7 years. In addition, in this study, corticosteroids were associated with fivefold increase of *in situ* anal cancer (Madeleine *et al*, 2013). Cyclosporine and azathioprine were related to increased incidence of invasive anal cancers, and tacrolimus and mycophenolate with a decreased incidence (Madeleine *et al*, 2013). Although, HPV-related precancerous lesions and cancers seem to be associated with immunosuppression and not organ related, because different organ-transplantation are associated with different immunosuppressive regimens, different rates of ASIL might be expected. Liver transplantation is the one of the most common solid-organ transplantations worldwide (second to kidney), underlining the relevance of studying this setting. Smoking was associated with ASIL in several studies (Etienney *et al*, 2008; Schwartz *et al*, 2013; Melo *et al*, 2014) and this was also confirmed in our analysis. A history of anal receptive intercourse is recognised as a risk factor for ASIL in men and so this was an exclusion criterion for both groups, but data are conflicting in women. A similar study in kidney transplant recipients also did not consider anal sex in women an exclusion criterion for ASIL prevalence analysis (Ogunbiyi *et al*, 1994).

Our results are in accordance with related published data that liver transplant recipients have a higher rate of cervical cytology abnormalities and anal HPV infection rate. A 20% prevalence of abnormal cervical cytology in early postoperative period after liver transplantation was described (Grat *et al*, 2017). A study by Roka *et al* (2004) analysed the prevalence of anal HPV infection in liver and kidney recipients before starting immunosuppressive therapy (anal cytology were obtained within 24 h after transplantation). HPV infection in liver patients was 29.4% (higher than in kidney transplants 29.4% vs 20.9%) and 17.6% were high-risk HPV. In a study by Grat *et al* (2014), HPV prevalence was 18% (high-risk 8% and low-risk 10%) in the first 3 weeks after liver transplantation. Anal HPV infection was higher in patients with hepatitis B and with  $\geq 3$  sexual partners and hepatitis B infection was the only factor associated with high-risk HPV infection. No ASIL prevalence was determined by anal cytology or HRA in both of these anal HPV studies.

Solid organ transplant recipients have a higher risk of HPV-related malignancies (Grulich *et al*, 2007; Madelaine *et al*, 2013). A study by Madelaine *et al* (2013) showed that solid organ transplant recipients had significantly elevated incidence of *in situ* and invasive HPV-related cancers at all sites (anus, penis, vagina, vulva and oropharynx), except for invasive cervical cancer. This was probably due to an effective and successful screening strategy and suggested the need for screening of other HPV-related cancers.

Anal squamous intraepithelial lesions are normally only detected with magnification and after acetic acid application they are not routinely accessible to touch (except if a cancer is already present); thus, regular digital ano-rectal examinations as the only screening method may be of limited value.

The major strengths of this study include successful HRA performance in an experienced centre in every patient with cytological abnormalities, the existence of a control group and the strict inclusion/exclusion criteria that were applied, thus ensuring that only immunosuppression in the post-transplant setting was being evaluated as a risk factor for ASIL.

Our study had some limitations and one of them is that the lack of HPV testing. This was not performed because information on HPV infection in the liver transplant patient has been previously published in two studies (Roka *et al*, 2004; Grat *et al*, 2014). Our aim was to specifically address ASIL prevalence. In the kidney transplant setting, anal HPV testing did not help to detect patients with ASIL (Ogilvie *et al*, 2008), but results are conflicting (Patel *et al*, 2010). More data, especially on the possible role of HPV genotyping in follow-up interval determination and risk stratification are required. Although the sample size for our study seems small, an initial statistical analysis found the number to be adequate. Owing to our sample size, conclusions related to the duration and type of immunosuppression impact in ASIL may be limited. There was a small difference encountered in the mean age of the groups (control group  $59 \pm 11$  years vs transplant  $54 \pm 11$  years), probably because control subjects (slightly older) were mostly colonoscopy referrals. Anal cytology is not the ideal screening method. The sensitivity ranged from 69% to 93% and specificity from 32% to 59% in a 2006 systematic review including HIV positive individuals (Chiao *et al*, 2006). Although HRA is considered the 'gold standard', this technique is not routinely recommended as a screening test because it is invasive, more expensive and very few centres have trained clinicians performing it. Thus, anal cytology has been favoured as an initial screening test (Alam *et al*, 2016) in high-risk groups (such as transplant recipients), followed by HRA in those with abnormal results. We referred all abnormal cytological results to HRA, because even patients with cytological diagnoses of ASC-US and LSIL could be diagnosed with HSIL at HRA/biopsy. High-resolution anoscopy was not performed in every patient and this raises the possibility of having patients with normal anal cytological results (therefore not submitted to HRA) and ASIL (underestimation of the true prevalence of HSIL). The negative predictive value of anal cytology is high in comparison with HRA/histology (Jin *et al*, 2016) and the possibility of having false negatives and underestimation of the disease is small.

Only patients with over 2 years of transplant were included; thus, the higher prevalence of lesions detected and the subsequent indication for screening based on this can only be extrapolated to this post-transplant period. In this study, the only focus was screening and only one sample of each of these patients was obtained. There is also a need for more data in the post-transplant setting that can complement this information, namely the performance of anal cytology and HRA in the follow-up period and the ideal follow-up intervals for this group.

HPV vaccination remains an open topic in transplantation. Vaccination ideally before transplantation should be considered. The quadrivalent HPV vaccine in the post-transplant period in adult patients seems to have suboptimal immunogenicity, especially early after transplant and in patients with a higher tacrolimus levels (Kumar *et al*, 2013). The rate of seropositivity only ranged from 52.6% to 68.4% depending on the HPV type (Kumar *et al*, 2013). The vaccine showed favourable safety and tolerability in this setting (Kumar *et al*, 2013). Further studies on HPV vaccination in transplanted patients are needed.

## CONCLUSIONS

This study brings new and relevant information to an unexplored group and tries to overcome some of the most important limitations from previous kidney transplant studies. Recognition of high-risk groups, recommendations for screening anal precancerous lesions and prevention (like HPV vaccination) are urgently needed for improving patient care. Current anal cancer projections show that this is more relevant than ever. Liver transplant patients have a higher risk of ASIL and screening should be considered, especially in smokers.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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