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De novo colorectal cancer after liver and kidney transplantation–Microenvironment disturbance

Rui Caetano Oliveira[a,b,c,d,*], Edgar Tavares-Silva[b,c,e], Ana Margarida Abrantes[b,c,g], Hugo Antunes[b,c,e], Paulo Teixeira[a], Ana Gomes[a], Ricardo Martins[b,c,f,g,h], Emanuel Furtado[g], Arnaldo Figueiredo[c,e], Beatriz Costa[c,f], Maria Augusta Cipriano[a], José Guilherme Tralhão[b,c,f,g,h], Maria Filomena Botelho[b,c,g]

[a] Pathology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[b] Biophysics Institute, Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[c] Coimbra Institute for Clinical and Biomedical Research (iCBR) area of Environment Genetics and Oncobiology (CIMAGO), Faculty of Medicine, University of Coimbra, Coimbra, Portugal
[d] Faculdade de Medicina da Universidade do Porto, Porto, Portugal
[e] Urology Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[f] Surgery Department, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[g] CNC.IBILI Consortium/CIBB, University of Coimbra, Coimbra, Portugal
[h] Liver Transplantation Unit, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal

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ABSTRACT

Colorectal cancer (CRC) is a major health burden and may arise as a complication of solid organ transplantation. Our study aimed to assess the incidence of the CRC in kidney and liver transplanted patients at a tertiary and reference center and to describe their clinical and pathological features.

Twelve patients, 10 men and two women, with a mean age of 60 years, composed our cohort, ten of them submitted to CRC resection. Transplanted organ was liver in five patients and kidney in seven. Regarding overall survival, patients submitted to renal transplantation were all deceased 5 years after CRC diagnosis, while those subjected to hepatic transplantation had a survival of 60% at the fifth year.

Pathology examination showed seven patients with advanced disease (stage III/IV) and high amount of necrosis. Tumor microenvironment was disturbed, with low inflammatory infiltrate, absence of natural killer cells and no PD-L1 expression. CRC exhibited microsatellite instability in 40%, with expression of cancer stem cell markers (CD133, CD44 and ALDH1), as well as P53 (50%) and KRAS mutations (41.7%).

CRC cancer after kidney and hepatic transplantation is a rare, but aggressive and deadly event. Regular follow-up should be instituted in these patients.

1. Introduction

Colorectal cancer (CRC) is a major health concern, being the third most common cancer type, accounting for 10.2% of the total cancer incidence, and the second most common cause of cancer-related death [1] In spite of all the improvement in clinical and biological knowledge, with appropriate treatment and prevention strategies, CRC remains an important issue [2], and the mortality rate related to this type of cancer is increasing [1,3].

Risk factors for CRC were well identified and go from environment to genetics [4] and the pathophysiology of CRC is widely studied with recognition of four genetic pathways: chromosomal instability, mutations of DNA mismatch repair genes, proto-oncogene and PI3K pathways [5].

Solid organ transplantation (SOT) has some risks, being malignancy one of the leading causes of death, especially hematopoietic tumors [6], most of them associated with activation of oncogenic viruses due to immunosuppression [7].

In recent years, immunosuppression has been advocated to provide increased risk of CRC, especially when induced by medication[8], due not only to diminished activity of the immune system [9] but also to opposition of the immunosuppressive drugs to chemotherapy agents

* Corresponding author.
E-mail address: ruipedrocoliveira@hotmail.com (R.C. Oliveira).

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[10].

The aim of this study was to assess the incidence of the CRC in kidney and liver transplanted patients at our institution – Centro Hospitalar e Universitário de Coimbra (CHUC), a tertiary and reference center and to describe their clinical and pathological features.

2. Material and methods

Clinical and pathological review of patients with CRC diagnosis at CHUC, between January 2004 and December 2016, which were previously transplanted at the same institution (liver and kidney transplantation). Inclusion criteria were age over 18 year's old and histological confirmation of CRC after transplantation. Patients with CRC before transplantation were excluded.

2.1. Study population

The study included 12 patients, 10 men and 2 women, with a mean age of 60.54 ± 13.41 years (range 34–78); only three patients had age inferior to 50 years (25%). One patient underwent to both liver and kidney transplantation but since liver transplantation occurred first, it was considered in the liver transplantation cohort for this research purposes.

Colorectal carcinoma management, including neoadjuvant and adjuvant treatments (radiotherapy and chemotherapy) and follow-up are accomplished in consonance with the European Society for Medical Oncology (ESMO) recommendations, National Comprehensive Cancer Network (NCCN) and American Society of Colon and Rectal Surgeons (ASCRS).

2.2. Pathological analysis

Patients' material was reviewed with analysis of CRC tissue available in paraffin blocks from surgical specimens. The samples were observed after a routine staining with hematoxylin and eosin (H&E). The histopathologic review was blinded, without previous knowledge of patient's data (such as type of transplantation) or outcome.

Histological growth pattern was defined according to the criteria of Koelzer and Lugli [11] as: infiltrative tumor growth (dissection of the bowel wall structures by tumor tissue with little or absent desmoplastic stromal response) and pushing tumor growth (clear demarcation of the tumor invasive front and host tissue). Due to the necessity of to observe the tumor/host interaction, this characteristic was only possible to evaluate on surgical specimens.

Tumor necrosis was assessed according to Schneider and Langner [12]: focal necrosis (<10% of the tumor area), moderate (10–30% of tumor area) and extensive (>30% of the tumor area). The inflammatory response was also evaluated at the tumor invasive border with the Klintrup's criteria [13]: low grade (absence or mild inflammatory infiltrate) and high grade (moderate or severe inflammatory infiltrate, with tumor destruction). Tumor-associated eosinophils were assessed as reported by Fernández-Aceñero et al. [14]: absence of eosinophils – grade 0, low eosinophil counts (<10/HPF) – grade 1, intermediate eosinophil counts (10 –50/HPF) – grade 2, and high eosinophil counts (>50/HPF) – grade 3. The evaluation was only done in surgical specimens.

Microsatellite instability (MSI) was tested according to the guidelines defined by the ESMO Clinical Guidelines for familial risk-colorectal cancer [15]: a immunohistochemical panel with MLH1, MSH2, MSH6 and PMS was primary used, and if there was loss of MLH1, subsequent immunohistochemical testing for BRAF is performed. The MSI evaluation was performed on all the patients.

P53 immunohistochemical analysis was performed to infer P53 mutation. An expression of P53 in more than 75% of tumor cells was considered overexpression and indicator of P53 mutations, as described by Akshatha et al. [16].

The presence of cancer stem cells (CSCs) was assessed with imunohistochemical staining for CD44, CD133 and ALDH1. It was considered a high number of CSCs when one of the markers was expressed in more than 50% of the tumor cells.

The presence of natural killer (NK) cells was evaluated as stated by Schonoccia et al. [17]: number of NK cells were counted per mm^2 and tumors were considered positive when there were more than 4 NK cells/mm^2. The evaluation was only done in surgical specimens.

Programmed death-ligand 1 (PD-L1) expression was graded according to the guidelines established for non-small cell lung cancer [18–20]: absence of staining – score 0; membranous and/or cytoplasmic staining >1% and <50% of the tumoral cells – score 1; staining in ≥50% of the tumoral cells – score 2. The staining was accomplished in all the patients.

Immunohistochemistry studies were performed on one 4 μm thick tissue sections from a representative block of the tumor, in a Ventana Marker Platform Bench Mark ULTRA IHC/ISH, resorting to an indirect multimeric detection system, biotin-free and peroxidase conjugated, with the following antibodies: CD68 (KP1, Ventana, Tucson, AZ-USA), P53 (DO7, Ventana, Tucson, AZ-USA), PD-L1 (22C3, Dako, Denmark), CD44 (SP37, Ventana, Tucson, AZ-USA), CD133 (17A6.1, Millipore, Boston, MA-USA), ALDH1 (EP1933Y, AbCam, Cambridge, UK), MLH1 (M1, Ventana, Tucson, AZ-USA), MSH2 (G219-1129, Ventana, Tucson, AZ-USA), MSH6 (44, Ventana, Tucson, AZ-USA) and PMS2 (EPR3947, Ventana, Tucson, AZ-USA). Adequate controls for each antibody were used, according to manufacturer instructions in order to achieve the best signal-to-noise ratio.

All stained slides were observed under a light microscope—Nikon Eclipse 50i—and images were obtained using a Nikon-Digital Sight DS-Fi1 camera.

KRAS mutations were evaluated resorting to Polymerase Chain Reaction (PCR) on a 10 μm thick cut section of paraffin-embedded tumor ensuing an area of 25–300 mm^2 with a minimum of 20% of tumor cells. The genetic study was performed in all patients. If no KRAS mutation was detected, sequencing for NRAS and BRAF mutations were carried.

2.3. Statistical analysis

Metric variables were described by median ± standard deviation (SD), and mean values were compared using Student's t tests. Categorical variables were described by absolute and relative frequencies, and the distributions were compared using Chi square tests. A two-sided p value <0.05 was considered representative of statistical significance. Statistical calculations were performed with SPSS (Version 22.0, Chicago, IL).

3. Results

Twelve patients were identified, five previously subjected to hepatic transplantation and 7 to renal transplantation. Colorectal cancer was located at the right colon in five patients (41.7%) and in the left colon in seven patients (58.3%); no tumor was located in the rectum. Two (20%) patients did not undergo surgery for CRC – diagnosis confirmed by endoscopic findings and tumor biopsy. Right hemicolectomy was performed in five patients, left hemicolectomy in two, subtotal colectomy in two and total proctocolectomy in one. Regarding histological subtype, CRC were all low-grade adenocarcinomas, NOS, three of them with mucin production but comprising less than 50% of tumor volume (WHO 2010 Classification [21]). Three out of ten tumors did not have lymph node invasion; the remaining six had a mean of 4.67 ± 4.03 positive lymph nodes (range 1–11).

3.1. Liver transplantation cohort

Five patients had been previously submitted to liver transplantation

(period Jan/92-Dec/2016; 755 procedures – incidence of 1:151), all male, with a mean age of 53.4 ± 8.08 years at transplantation (range 43–62). Four (83.3%) were cirrhotic due to alcohol consumption and the remaining one (17.7%) was a secondary biliary cirrhosis due to primary sclerosing cholangitis (PSC). One of the alcohol induced cirrhosis patient had a well differentiated hepatocellular carcinoma (HCC), G1 by Edmonson [21], with 2 cm and without vascular invasion (TNM – T1) and other had a necrotic nodule with 4 cm without residual tumor (no records of previous treatment). The patient with PSC had also a medical record of ulcerative colitis (UC), without signs of dysplasia on previous endoscopy and biopsies.

Of note, the patient with the HCC was submitted to renal transplantation 8 months after liver transplantation, due to idiopathic kidney failure.

All liver donors were AB0 compatible and deceased, with a median age of 50 ± 8.23 years (range 44–65), three male and two females.

All patients underwent immunosuppression with prednisolone, tacrolimus, everolimus and mycophenolate mofetil.

CRC was diagnosed after a median of 55.2 ± 26.13 months (range 16–77) after liver transplantation; patients had a median age of 58 ± 10.19 years (range 44–69) – Fig. 1A.

Tumor was located in the right colon in two patients and in the left colon in three. Four patients were submitted to surgery. On gross examination, two were ulcerated and two were circumferential and stenosing. Two patients had advanced disease (Stage III/IV). Three CRC were infiltrative. Two had a low inflammatory infiltrate and absent/low number of eosinophils. Two CRC exhibited high necrosis.

The clinicopathological characteristics can be consulted on Table 1.

3.2. Kidney transplantation cohort

Seven patients had been previously submitted to kidney transplantation (period Jan/92-Dec/2016; total of 2662 procedures) – incidence of 1:380 patients; five were men (71.4%) and two women (28.6%) with mean age of transplantation of 52.75 ± 17.57 (range 24–70). None of the patients had smoking or alcoholic habits. Five patients had dyslipidemia, with one also affected by arterial hypertension. One patient had diabetes mellitus. All of them did dialysis before transplantation with a mean time of 45.43 ± 25.55 months (range 9–84).

Regarding etiology of renal failure: two chronic glomerulonephritis, two polycystic renal disease, and one was due to nephrotoxicity; two patients had renal failure of undetermined cause. All kidneys provided from a deceased male donor, AB0 compatible, with mean age 43.71 ± 17.59 years (range 27–57).

All patients were under immunosuppression in a mean of 10.86 ± 6.37 months (2–17) with combination of at least 3 agents: all were under prednisolone and two patients were also taking azathioprine and cyclosporine A; the remaining agents were rapamycin (one patient), tacrolimus (four patients), mycophenolate mofetil (two patients) and antithymocyte globulin (one patient).

None had records of Epstein Barr virus (EBV) infection and five had cytomegalovirus infection (CMV). Only two had record of acute rejection and a different patient had episode of chronic rejection.

CRC developed after a mean of 117.85 ± 76.97 months (range 14–197) after renal transplantation. At CRC detection patients presented a mean age of 61.43 ± 16.65 years (range 34–78). Tumors had a slight prevalence in the left colon vs. the right (4:3).

There was no statistical difference between the median ages of patients at the time of CRC development nor between the median time from transplantation and CRC in both groups.

Regarding overall survival (OS), all of the patients were dead with a mean survival of 135.28 ± 67.74 months after renal transplantation (range 36–204) and 117.86 ± 76.98 months after CRC diagnosis (range 14–197) – Fig. 1B.

The clinicopathological characteristics can be consulted on Table 1.

3.3. Immunohistochemical analysis

a) Microsatellite instability

MSI assessment revealed loss of expression for DNA mismatch repair proteins in two patients from the hepatic transplantation cohort (33.3%) and in two patients from the renal transplantation group (28.6%). There was isolated loss of MSH6 in two patients, loss of MSH2 an MSH6 in one and loss of PMS2 in one patient – Fig. 2A.

b) P53 mutation status

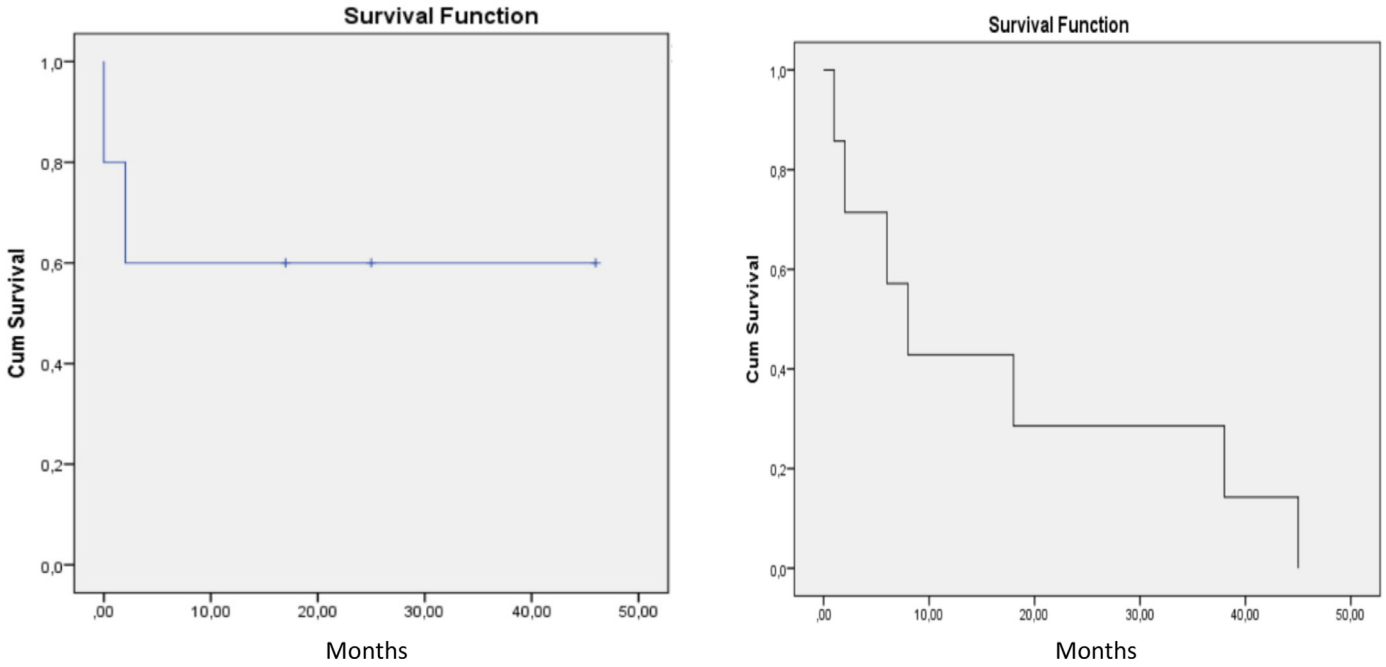

Fig. 1. Kaplan-Meyer survival curves for the hepatic transplantation cohort (A) and renal transplantation cohort (B).

Table 1
Clinicopathological characteristics of the patients of the hepatic and renal transplantation cohorts.

	Hepatic transplantation cohort	Renal transplantation cohort
Number of patients	*N* = 5	*N* = 7
Median age at transplantation ± standard deviation (years)	52 ± 8.08	52.75 ± 17.57
Gender		
Male	5	5
Female		2
Previous history of ulcerative colitis		
No	4	–
Yes	1	
Median time of CRC development after transplantation (months) ± standard deviation	55 ± 10.19	117.85 ± 76.97
Median age at CRC detection ± standard deviation (years)	55.2 ± 26.13	61.43 ± 16.65
Tumor location		
Right colon	2	3
Left colon	3	4
Number of patients with surgical CRC resection	4	6
Pathological staging (AJCC)		
Stage II	2	1
Stage III/IV	2	5
Growth pattern		
Infiltrative	3	6
Expansive	1	0
Lymph node metastases		
No	2	2
Yes	2	4
Inflammatory infiltrate		
Low grade	2	3
High grade	2	3
Number of Eosinophils		
Low/Absent	2	6
Intermediate/High	2	0
Necrosis		
Focal/Intermediate	2	1
High	2	5
Microsatellite instability		
Unstable	2	2
Stable	3	5
P53 status		
Wild type	2	4
Mutated	3	3
Cancer stem cells overexpression		
CD44	3	4
ALDH1	4	5
CD133	5	7
Natural killer (NK) cells		
Negative	4	6
Positive	0	0
PD-L1 expression		
Negative	5	7
Positive	0	0
RAS status		
Wild-type	2	5
Mutated	3	2

P53 mutations, assessed by immunohistochemistry, was detected in three (60%) patients of the hepatic transplantation group and in three patients (42.9%) of the renal transplantation cohort. An example of P53 overexpression can be seen in Fig. 2B.

c) Cancer stem cells expression

CRC from both groups revealed high expression of cancer stem cells markers with all tumors overexpressing CD133; and high expression of CD44 (Fig. 2C) in 60% and 57.1%; and ALDH1 in 80% and 71.4%, at the hepatic and renal transplantation cohort respectively.

d) NK cells assessment and PD-L1 expression

In all tumors there were no identifiable NK cells (CD56 positive), with both external and intrinsic (neural components) positive controls – Fig. 2D.

Regarding to PD-L1, none of the CRC specimens exhibited expression – score 0, both in tumor cells and tumor-infiltrating immune cells.

3.4. KRAS evaluation

Genetic study showed mutation on KRAS gene in three patients of the hepatic transplantation cohort (60%) and in two patients from the renal transplantation cohort (28.6%). In the non-RAS mutated patients, genetic study also included NRAS and BRAF evaluation which was negative.

The imunohistochemical and genetic results, together with the clinical and pathological data can be consulted on Table 1.

4. Discussion

Our results confirm that CRC after solid organ transplantation is a real challenge and has high mortality, as stated by our results with all the patients in the renal transplantation cohort dead after eight months (median time) after CRC diagnosis. Our cohorts had an increased incidence – 1:151 in the hepatic transplantation cohort and 1:380 in the renal transplantation cohort, when compared with the general population [22,23]. Our patients presented with a median age inferior at the time of CRC detection, namely 55 and 61 years in the hepatic and renal transplantation cohort respectively, against the average of 67 years-old stated in seer.cancer.gov [24] (accessed in February/2019).

Concerning CRC location, our cohorts exhibited a slight prevalence for the left side of the colon; however, the low number of patients does not allow taking conclusions. On a histological level, tumors had characteristics of aggressive biological behavior with high amount of necrosis in seven of the ten patients subjected to surgery, which has been appointed as an indicator of poor prognosis in CRC [25]. Also the vast majority of patients presented with advanced disease, namely stage III/IV in seven out of ten patients, and nine had a infiltrative growth pattern, associated with high tumor budding and tissue disruption and, consequently, worse prognosis, as described by Koelzer et al. [11].

Tumor microenvironment showed that in 50% of the tumors, the inflammatory infiltrate was of low grade and in the majority of the patients (8 out of 10) the number of eosinophils was low or even absent. These findings are in consonance with aggressive tumors, since a high number of eosinophils is usually associated with a microenvironment able of tumor modulation [26]. The density of the inflammatory infiltrate is also a good prognostic biomarker for favorable survival, with longer overall survival and disease-free survival in patients with more dense inflammatory infiltrate in the stroma [27]. Our patients presented with scarce inflammation, in relation with immunosuppressive therapy and consequent microenvironment modulation.

In consonance with these findings, the number of NK cells in the microenvironment was also diminished with no immunohistochemically-detected cells in the microenvironment. NK cells have the ability of recognizing cancer cells and promote their destruction, especially the cancer promoting cells [28]. The ability of tumor cells to escape this mechanism of defense has been recognized as tumor promoting event and therapies for stimulating NK cells have been described as effective in CRC treatment [29–31]. In our cohorts, patients were depleted of NK cells, which may indicate that in these situations NK cells based therapies are not effective.

Still on the microenvironment level, our patients did not expressed PD-L1. PD-L1 has been recorded in the last years as a predictor marker for checkpoint inhibitor therapy in CRC [32]. PD-L1 is expressed by

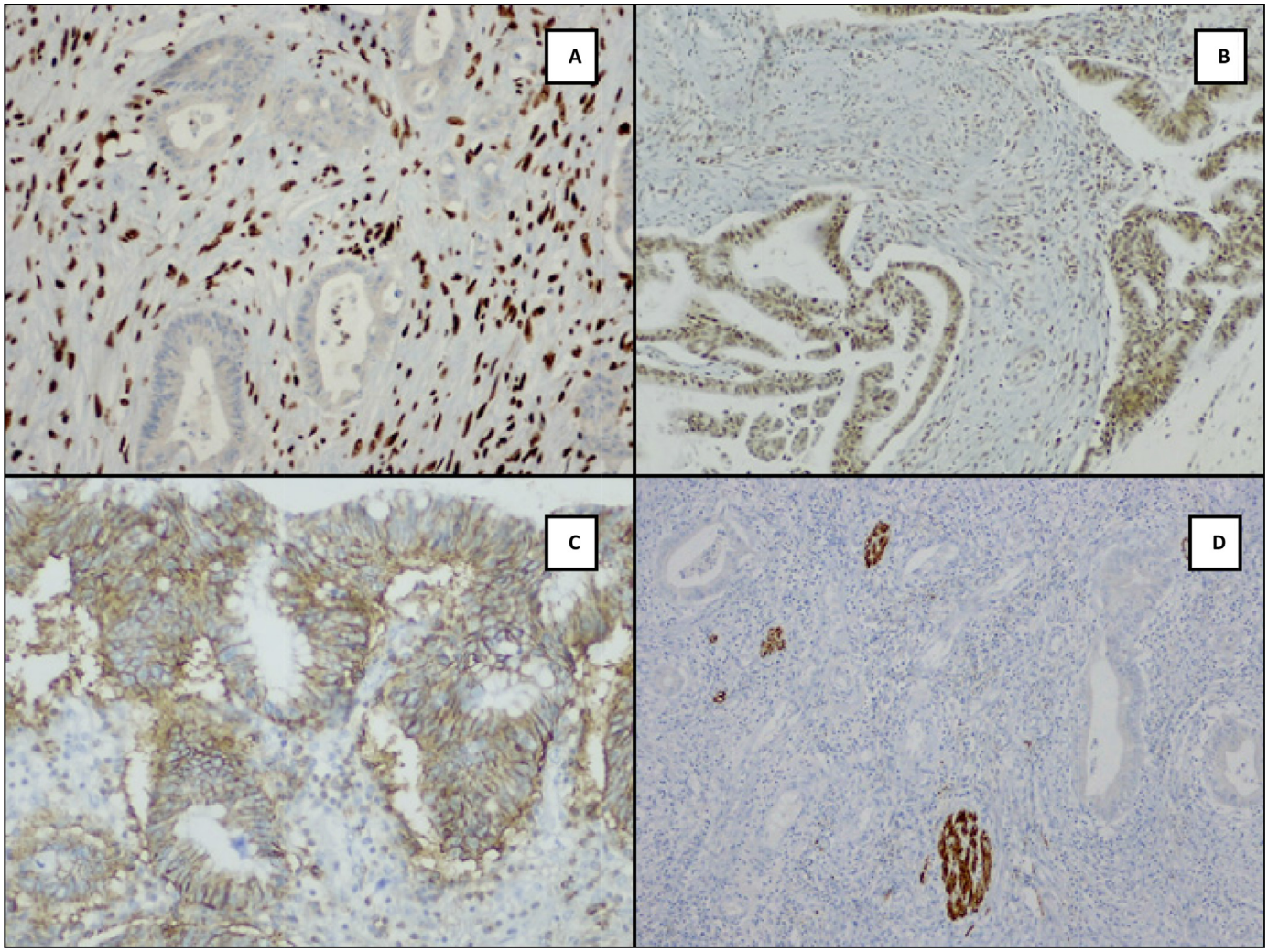

Fig. 2. Immunohistochemical studies: A – loss of MHS2 expression on tumor cells and positive staining in lymphocytes (internal control), 100x; B – overexpression of P53 in almost all the tumoral cells, 100x; C – High expression of CD44 in tumor cells, presenting as a distinct membrane pattern, 200x; D – no evidence of Natural Killer (NK) cells in the tumor microenvironment, assessed by CD56 immunostaining, with positivity in entrapped neural fibers (internal control), 100x.

tumor cells in order to suppress the PD1/PD-L1 axis, and overexpression of PD-L1 by immunohistochemistry is a good surrogate marker for checkpoints inhibitors that will provide the immune system a boost in its capacity to interact with the tumor cells and induce their destruction [33,34]. In patients under immunosuppression, as our patients, the immune system is on a low activity and every strategy is useful against cancer, but none of the tumors expressed PD-L1, not supporting this therapeutic approach. The immunosuppression is related with a low activity of the immune system, since in a low immune activity the tumor cell does not need to express PD-L1; supporting this theory is the fact that high levels of tumor infiltrating lymphocytes are usually associated with upregulation and overexpression of PD-L1 [35]. Nevertheless, even in patients without expression of PD-L1, therapy with checkpoint inhibitors may be possible, especially if other characteristics are present, namely IMS and high tumor mutational burden [36].

Regarding tumor proprieties, there was a relatively high frequency of MSI – 40%, when compared to the 10–15% reported on the literature on CRC [37]. This may represent a selection bias or may represent a subgroup of CRC defined by molecular characterization [38], which may benefit from a immunosuppression status. Nevertheless, the MSI status may provide a therapeutic window with anti-PD-L1 agents, even in the absence of PD-L1 expression [33,35,39]. An association with Lynch syndrome in the patients was excluded by consultation of the clinical information.

The CRC also overexpressed cancer stem cells markers, as assessed by immunohistochemical evaluation of CD133, CD44 and ALDH1. Cancer stem cells are a well-known factor of tumor aggressive potential and resistance to therapy [40–43]. The overexpression in these particular cohorts may be related with a deficient microenvironment that was not able to keep them under control with subsequent expansion.

P53 evaluation showed mutation in 50% of the patients, which was in agreement with the reported in literature [44], also representing a more aggressive behavior and resistance to conventional therapies [45]. Finally, concerning KRAS status, we found mutations in five patients (41.7%), also in accordance with the literature [46]; KRAS mutations are routinely screened in patients with advanced CRC since they predict resistance to anti-EGFR agents and are associated with more aggressive course of disease [47].

The study has limitations, namely the retrospective review of patients submitted to liver and kidney transplantation that subsequently developed CRC and the fact that it was performed in a single institution. Some patients that were transplanted in our institution are followed in other hospitals and we lost record of them, meaning that our cohort could even be bigger. However, there are not many single institutions cohorts regarding this subject and, in case of liver transplantation, revealed a focus on patients with PSC which represent a very specific sub cohort of patients, usually with UC associated CRC cancer [8,48]. The

reported studies in the literature exhibited an incidence and number of patients similar to ours cohort [48–54], however they only report incidence, cancer location and staging, without approaching a detailed classification of the tumor, namely MSI status, P53 mutations, presence of cancer stem cells, and the tumor microenvironment characterization.

In conclusion, CRC after renal and hepatic transplantation is an aggressive disease, with high mortality, and due to low activity of the immune system, some therapies are limited. A better understanding of the microenvironment may allow us to tailor some patients for individualized therapies. Despite being infrequent, the transplanted patient is a major investment of the health care system and efforts should be made in order to improve their lifetime and diminish the associated risks of transplantation. At the moment, the best option seems to be a close follow-up of the patients with regular colonoscopies allowing early detection and intervention.

Authors' contribution

RCO and MAC designed the study, provided histological analysis and wrote the manuscript. HA, ETS and RM analyzed clinical records of transplanted patients (renal and hepatic). PT performed immunohistochemical studies. AMA was responsible for the statistical description. AG performed sequencing for RAS and BRAF mutations. EF and AF supported clinical records consultation. BC performed manuscript revision. JGT was a consultant regarding surgical aspects and interest of the study and performed critical review of the manuscript. MFB performed critical revision and added content to the manuscript.

All authors have read and approved the final version of the manuscript.

Declaration of Competing Interest

There is no conflict of interest.

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References

[1] F Bray, et al., Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries, CA. Cancer J. Clin. 68 (2018) 394–424.
[2] F. Grizzi, G. Di Caro, F. Marchesi, L. Laghi, Prognostic value of innate and adaptive immunity in cancers, in Cancer Immunology: A Translational Medicine Context, 2015, https://doi.org/10.1007/978-3-662-44006-3_15.
[3] J Ferlay, et al., Cancer incidence and mortality worldwide : sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer (2012), https://doi.org/10.1002/ijc.29210.
[4] C.M Johnson, et al., Meta-Analyses of colorectal cancer risk factors, Cancer Causes Control (2013), https://doi.org/10.1007/s10552-013-0201-5.
[5] S. Prenner, J. Levitsky, Comprehensive review on colorectal cancer and transplant, Am. J. Transplant. (2017), https://doi.org/10.1111/ajt.14340.
[6] E.A Engels, et al., Spectrum of cancer risk among US solid organ transplant recipients, JAMA - J. Am. Med. Assoc. (2011), https://doi.org/10.1001/jama.2011.1592.
[7] E.A. Mesri, M.A. Feitelson, K Munger, Human viral oncogenesis: a cancer hallmarks analysis, Cell Host Microbe (2014), https://doi.org/10.1016/j.chom.2014.02.011.
[8] M Safaeian, et al., Risk of colorectal cancer after solid organ transplantation in the united states, Am. J. Transplant. (2016), https://doi.org/10.1111/ajt.13549.
[9] T.F. Gajewski, H. Schreiber, Y.X. Fu, Innate and adaptive immune cells in the tumor microenvironment, Nat. Immunol. (2013), https://doi.org/10.1038/ni.2703.
[10] I Yokoyama, et al., Enhancement of tumor proliferation by cyclosporine a in early phase of experimental hepatic metastasis, Jpn J. Cancer Res. (1994), https://doi.org/10.1111/j.1349-7006.1994.tb02418.x.
[11] V.H. Koelzer, A. Lugli, The tumor border configuration of colorectal cancer as a histomorphological prognostic indicator, Front. Oncol. (2014), https://doi.org/10.3389/fonc.2014.00029.
[12] N.I. Schneider, C. Langner, Prognostic stratification of colorectal cancer patients: current perspectives, Cancer Manag. Res. (2014), https://doi.org/10.2147/CMAR.S38827.
[13] K Klintrup, et al., Inflammation and prognosis in colorectal cancer, Eur. J. Cancer (2005), https://doi.org/10.1016/j.ejca.2005.07.017.
[14] M.J. Fernández-Aceñero, M. Galindo-Gallego, J. Sanz, A. Aljama, Prognostic influence of tumor-associated eosinophilic infiltrate in colorectal carcinoma, Cancer (2000), https://doi.org/10.1002/(SICI)1097-0142(20000401)88:7<1544::AID-CNCR7>3.0.CO;2-S.
[15] J. Balmaña, F. Balaguer, A. Cervantes, D. Arnold, Familial risk-colorectal cancer: ESMO Clinical Practice Guidelines †, Ann. Oncol. 24 (2013) 73–80.
[16] A. Akshatha C, et al., Correlation of p53 Overexpression with the Clinicopathological Prognostic Factors in Colorectal Adenocarcinoma, J. Clin. Diagn. Res. 10 (2016) EC05–EC08.
[17] G Sconocchia, et al., NK cells and T cells cooperate during the clinical course of colorectal cancer, Oncoimmunology 3 (2014) e952197.
[18] G.L. Riely, What, when, and how of biomarker testing in non-small cell lung cancer, J. Natl. Compr. Canc. Netw. 15 (2017) 686–688.
[19] I.A Cree, et al., PD-L1 testing for lung cancer in the UK: recognizing the challenges for implementation, Histopathology 69 (2016) 177–186.
[20] E.-.E Paulsen, et al., Assessing PDL-1 and PD-1 in non–small cell lung cancer: a novel immunoscore approach, Clin. Lung Cancer 18 (2017) 220–233 e8.
[21] N.J. Carr, L.H. Robin, WHO Classification of Tumors of the Digestive System, fourth ed., IARC, Lyon, 2010, https://doi.org/10.6061/clinics/2018/e499 2010.
[22] J Ferlay, et al., Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012, Eur. J. Cancer 49 (2013) 1374–1403.
[23] M Arnold, et al., Global patterns and trends in colorectal cancer incidence and mortality, Gut (2017), https://doi.org/10.1136/gutjnl-2015-310912.
[24] No Title. Available at: https://seer.cancer.gov/.
[25] S.A Väyrynen, et al., Clinical impact and network of determinants of tumour necrosis in colorectal cancer, Br. J. Cancer 114 (2016) 1334–1342.
[26] A.L. Saraiva, F. Carneiro, New insights into the role of tissue eosinophils in the progression of colorectal cancer: a literature review, Acta Med. Port. 31 (2018) 329.
[27] K. Jakubowska, W. Kisielewski, L. Kańczuga-Koda, M. Koda, W. Famulski, Diagnostic value of inflammatory cell infiltrates, tumor stroma percentage and disease-free survival in patients with colorectal cancer, Oncol. Lett. 14 (2017) 3869–3877.
[28] R Tallerico, et al., Human NK cells selective targeting of colon cancer-initiating cells: a role for natural cytotoxicity receptors and MHC class I molecules, J. Immunol. 190 (2013) 2381–2390.
[29] A Coppola, et al., NK cell inflammation in the clinical outcome of colorectal carcinoma, Front. Med. 2 (2015) 33.
[30] H Zhao, et al., Natural killer cells inhibit oxaliplatin-resistant colorectal cancer by repressing WBSCR22 via upregulating microRNA-146b-5p, Am. J. Cancer Res. 8 (2018) 824–834.
[31] L Li, et al., Adoptive transfer of natural killer cells in combination with chemotherapy improves outcomes of patients with locally advanced colon carcinoma, Cytotherapy 20 (2018) 134–148.
[32] R Cantero-Cid, et al., PD-L1/PD-1 crosstalk in colorectal cancer: are we targeting the right cells, BMC Cancer 18 (2018) 945.
[33] Marginean, E.C. & Melosky, B.Is There a Role for Programmed Death Ligand-1 Testing and Immunotherapy in Colorectal Cancer with Microsatellite Instability? https://doi:10.5858/arpa.2017-0041-RA.
[34] A.M Valentini, et al., PD-L1 expression in colorectal cancer defines three subsets of tumor immune microenvironments, Oncotarget 9 (2018) 8584–8596.
[35] M Yi, et al., Biomarkers for predicting efficacy of PD-1/PD-L1 inhibitors, Mol. Cancer 17 (2018) 129.
[36] T.A Chan, et al., Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic, Ann. Oncol. 30 (2019) 44.
[37] H. Kawakami, A. Zaanan, F.A. Sinicrope, Microsatellite instability testing and its role in the management of colorectal cancer, Curr. Treat. Options Oncol. 16 (2015) 30.
[38] J Guinney, et al., The consensus molecular subtypes of colorectal cancer, Nat. Med. (2015), https://doi.org/10.1038/nm.3967.
[39] H Sato, et al., DNA double-strand break repair pathway regulates PD-L1 expression in cancer cells, Nat. Commun. 8 (2017) 1751.
[40] P. Dhawan, R. Ahmad, A.S. Srivastava, A.B Singh, Cancer stem cells and colorectal cancer: an overview, Curr. Top. Med. Chem. 11 (2011) 1592–1598.
[41] S. Roy, Cancer stem cells in colorectal cancer: genetic and epigenetic changes, J. Stem Cell Res. Ther. (2013), https://doi.org/10.4172/2157-7633.S7-006.
[42] R.C Langan, et al., Colorectal cancer biomarkers and the potential role of cancer stem cells, J. Cancer (2013), https://doi.org/10.7150/jca.5832.
[43] W Gao, et al., Isolation and phenotypic characterization of colorectal cancer stem cells with organ-specific metastatic potential, Gastroenterology (2013), https://doi.org/10.1053/j.gastro.2013.05.049.
[44] A. Fariña Sarasqueta, et al., Integral analysis of p53 and its value as prognostic factor in sporadic colon cancer, BMC Cancer 13 (2013) 277.
[45] X.-.L. Li, J. Zhou, Z.-.R. Chen, W.-.J. Chng, P53 mutations in colorectal cancer - molecular pathogenesis and pharmacological reactivation, World J. Gastroenterol. 21 (2015) 84–93.
[46] C.P. Vaughn, S.D. ZoBell, L.V. Furtado, C.L. Baker, W.S. Samowitz, Frequency of KRAS, BRAF, and NRAS mutations in colorectal cancer, Genes Chromosom. Cancer 50 (2011) 307–312.
[47] M. Porru, L. Pompili, C. Caruso, A. Biroccio, C. Leonetti, Targeting KRAS in metastatic colorectal cancer: current strategies and emerging opportunities, J. Exp. Clin. Cancer Res. 37 (2018) 57.
[48] M.A Mouchli, et al., Risk factors and outcomes of de novo cancers (excluding nonmelanoma skin cancer) after liver transplantation for primary sclerosing cholangitis, Transplantation 101 (2017) 1859–1866.
[49] A Merchea, et al., Outcomes of colorectal cancer arising in solid organ transplant

recipients, J. Gastrointest. Surg. 18 (2014) 599–604.
[50] E. Hall, D. Segev, E. Engels, Racial/ethnic differences in cancer risk after kidney transplantation, Am. J. Transplant. 13 (2013) 714.
[51] J.H Kwon, et al., Prevalence of advanced colorectal neoplasm after kidney transplantation: surveillance based on the results of screening colonoscopy, Dig. Dis. Sci. 60 (2015) 1761–1769.
[52] J.T Lee, et al., Colorectal surgery after kidney transplantation: characteristics of early vs. late posttransplant interventions, J. Gastrointest. Surg. 18 (2014) 1299–1305.
[53] T. Nishihori, M. Strazzabosco, M.W. Saif, Incidence and management of colorectal cancer in liver transplant recipients, Clin. Colorectal Cancer 7 (2008) 260–266.
[54] R.F. Saidi, P.S. Dudrick, M.H Goldman, Colorectal cancer after renal transplantation, Transplant. Proc. 35 (2003) 1410–1412.