De novo malignant melanoma occurred in renal allograft: DNA typing to determine the origin of the tumour

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Abstract: Malignant diseases are considered as great challenges in clinical transplantation. It is well known that the incidence of malignancy is higher in the transplanted population if compared with the normal population. It is important to distinguish between neoplastic diseases originating from pre-existing lesions in the transplanted organs and de novo graft tumours. Post-transplant malignancy of donor origin is a rare complication of organ transplantation, most likely transmitted as micrometastases within the parenchyma of the donor organ or from circulating tumour cells contained within the organ. Malignant melanoma, although its incidence is rather low, is one of the most common donor-derived tumour inadvertently transplanted, comprising 28% of donor transmitted tumours. Malignant melanoma in the graft without dermatological localisation is extremely rare. We report a case of de novo melanoma occurring in the allograft, where transmission from the donor was excluded by DNA (desoxyribonucleic acid) investigation. We did not find any data in the literature where a malignant melanoma occurred after transplantation in the transplanted kidney without any skin lesions and the donor origin was excluded. We draw attention to the importance of the DNA typing in case of tumours occurring in immunosuppressed patients.

Keywords: melanoma, de novo, kidney transplantation, tumour, transmission, DNA, typing

Introduction

Tumour development is an increasingly recognized problem in kidney transplanted patients. The incidence of malignancy in transplanted patients is higher than in the general population. Since the development of immunosuppressive therapy transplanted patients live longer, however, have greater chance to develop malignant diseases. Length and intensity of immunosuppressive therapy are related to the risk of post-transplant malignancy. The risk of developing malignancy is 14% at 10 years and 40% at 20 years after transplantation [1, 2].

The leading cause of death in kidney transplant recipients with a functioning graft are cardiovascular complications. Malignant tumours are the second most common cause of death after kidney transplantation [3, 4].

Malignant diseases are considered as great challenges in clinical transplantation [5, 6]. Skin tumours are frequently seen after organ transplantation, in particular, squamous cell carcinoma. The incidence of malignant melanoma is less extensively reviewed in the literature [7, 8].

In patients followed for 20 years after solid organ transplantation post-transplant malignancies occurred in up to 50 %. Post-transplant malignancies are expected to be the leading cause of death within the next 20 years in kidney transplant recipients. Cancer will surpass cardiovascular complications as the leading cause of death in transplant patients within the next 2 decades. It is important to distinguish between neoplastic diseases originating from pre-existing lesions in the transplanted organs and de novo graft tumours. An understanding of the underlying pathobiology and knowing how to minimize cancer risks in transplant recipients are essential. The etiology of post-transplant malignancy is believed to be multifactorial and involves impaired immunosurveillance of neoplastic cells as well as depressed antiviral immune activity with a number of common post-transplant malignancies being viral-related. Although calcineurin inhibitors and azathioprine have been linked with post-transplant malignancies, newer agents such as mycophenolate mofetil and sirolimus may even have antitumor properties [9, 10]. Long-term data evaluation is needed to determine

whether the use of these agents will ultimately diminish the mortality due to malignancy for transplant recipients. Early diagnosis and management of de novo malignant disease in transplant patients is crucial for the prognosis of graft function and patients survival [11–15].

Post-transplant malignancy of donor origin is a rare complication of organ transplantation, most likely transmitted as micrometastases within the parenchyma of the donor organ or from circulating tumour cells contained within the organ. The incidence of tumour transmission is reported to be 0.02–0.2%, with a mean time to diagnosis of 14.2 months [16–18]. Once a post-transplant malignancy is diagnosed, differentation of the malignancy is of recipient or donor derivation significantly influences the treatment algorithm. In addition to the withdrawal of immunosuppression, explantation and retransplantation may be the treatment protocol for donor transmitted malignancies. Malignant melanoma is one of the most common donor transmitted malignancy, comprising 28% of donor transmitted tumours [19–21].

We report a case of kidney transplanted patient who developed malignant melanoma in the transplanted kidney and the donor derived origin of tumour was excluded by DNA HLA typing.

Case report

The 23-year-old male with end stage renale failure due to focal segmental sclerosis was treated with regular haemodialysis until 1997, when a cadaveric donor kidney became available. The donor, a 45-year-old male had died of a massive intracerebral haemorrhage. The donor had no history or clinical evidence of melanoma or any other tumour, as confirmed by the donor procedure. Autopsy was not performed since no signs of malformation were known at the time of the kidney transplantation. There was a 1A, 1B and 1DR HLA (human leukocyte antigen) antigen match between the donor and recipient. DNA samples of the donor were stored (at minus 80 centigrade in liquid nitrogen). Both the donor and the recipient were CMV (cytomegalovirus) negative. The patient received cyclosporine and steroid-based immunosuppression. The postoperative course was uneventful, there were no rejection episodes and renal function was satisfactory

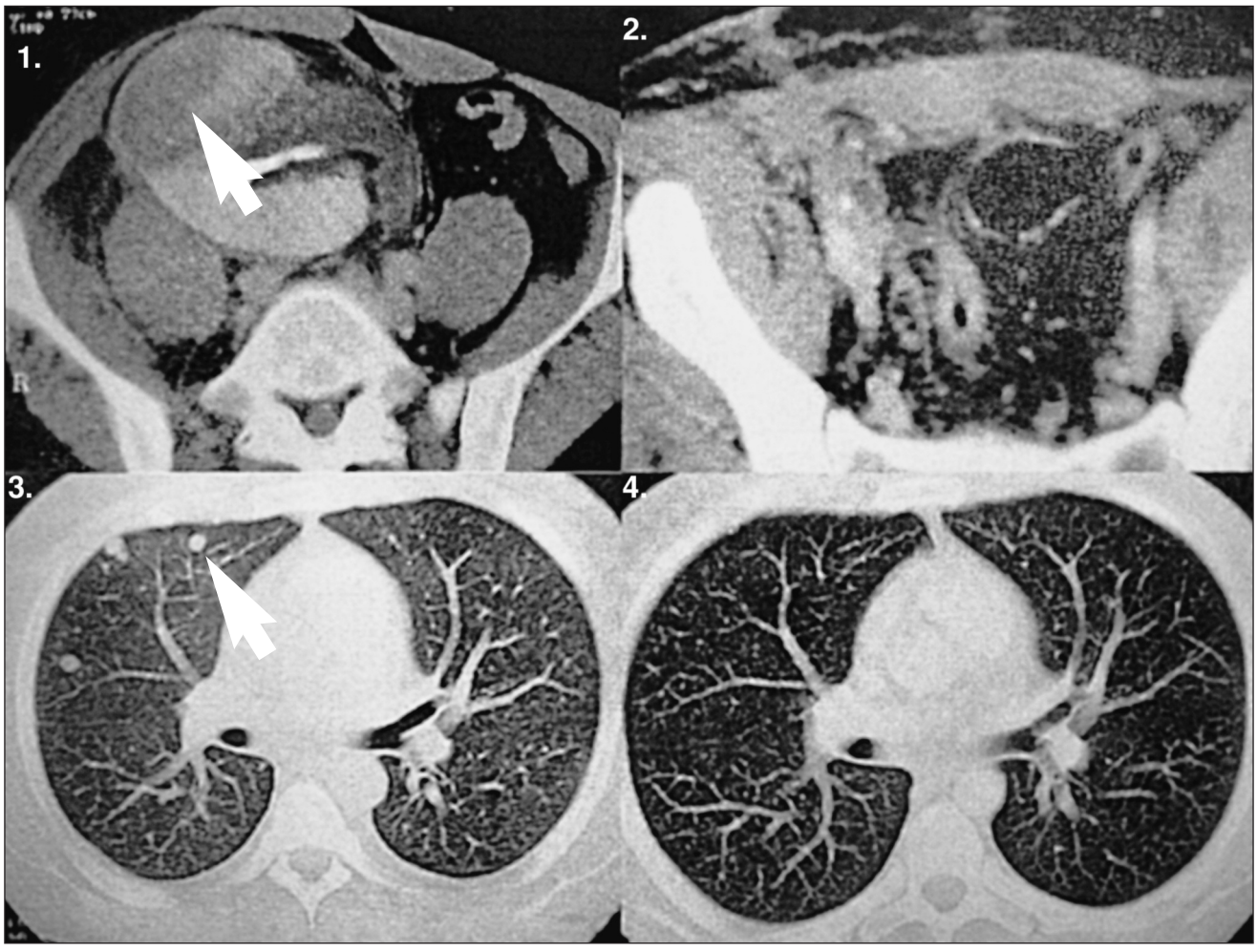

Fig. 1. CT scans of the patient, before and after chemotherapy CT scan of the minor pelvis (1.) with the tumourous kidney allograft in the right iliac fossa before (1.) and after (2.) removal. White arrow shows the malignancy. CT scan of the thorax with bilateral pulmonal metastases (3.), and a negative image after chemotherapy (4.). White arrows show the metastases

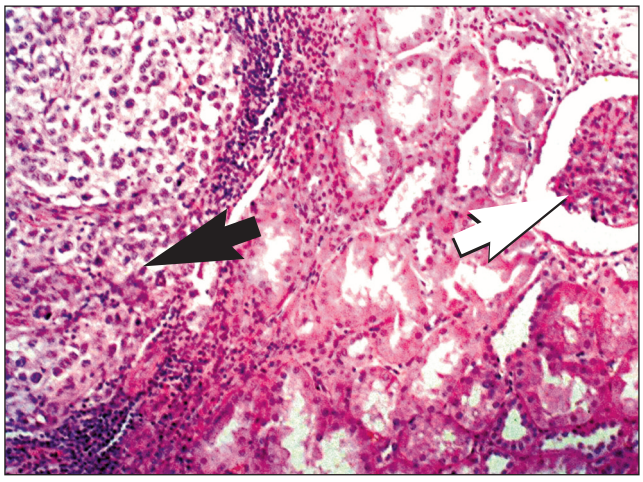

Fig. 2. HE staining, ×40 magnification. Black arrow shows the melanoma, white arrow points to a glomeruli

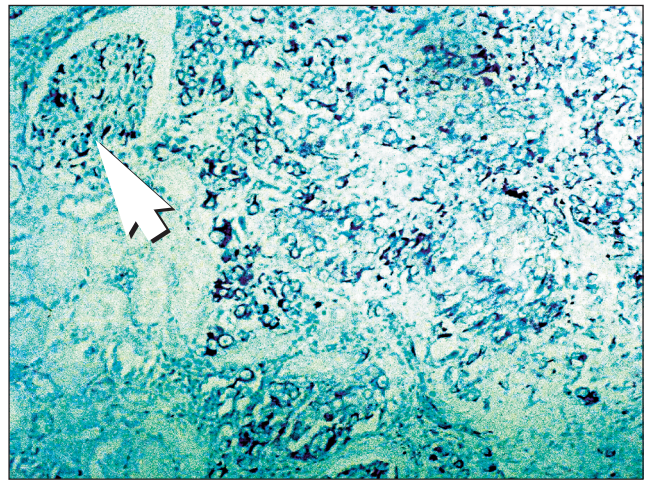

Fig. 3. S-100 protein immunostaining. ×40 magnification. Dark granules show positive staining for S-100 within all kidney structures. Also a glomeruli can be identified

(creatinine clearance of 80 ml/min) at discharge from hospital (18 days after transplantation). Thirteen months after the renal transplantation he was admitted to the hospital because of fever and weight loss. Renal function was unchanged; there were no signs of rejection. Ultrasonography of the graft showed a hyperdense, irregularly shaped tumour, with a diameter of 10 centimetres. Chest X-ray and CT (computer tomography) scan revealed multiple nodules, involving both lungs highly suspicious to be metastatic in origin (Fig. 1). The biopsy of the kidney graft showed a low-grade malignant mesenchymal tumour. Immunosuppression was withdrawn and graftectomy was planned. During the operation a large mass, infiltrating the whole kidney allograft, as well as the iliac vessels, the ureter and the abdominal muscles was found. The transplanted kidney was removed and a regional block dissection of the inguinal lymph nodes were performed. Regular haemodialysis was reinstituted.

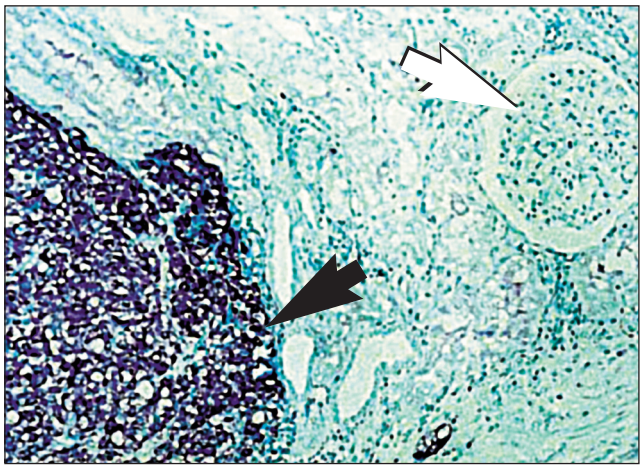

Fig. 4. HMB-45 staining ×40 magnification. Black arrow shows the melanoma invading the kidney. The tumour showes typical positive immunostaining. The white arrow points to the glomeruli

Upon macroscopic inspection the graft kidney showed several round foci of lobulated, partly necrotic tumour, the largest measuring 105 mm in maximum dimension. The tumour invaded the renal pelvis as well as other hilar structures.

On microscopic examination an invasive solid tumour could be identified (Figs 2 to 4). The cells showed remarkable atypical characteristics with frequent mitotic figures, swollen nuclei, prominent eosinophilic nucleoli, coarsely granulated chromatin pattern and occasionally vacuolated cytoplasm. Scattered multinucleated giant cells could also be demonstrated.

The immunohistochemistry was *positive* for vimetin, S-100 protein (Fig. 3), CEA (carcinoembryonic antigen), HMB-45 (Fig. 4) and was *negative* for LCA (leukocyte common antigen), CD31, cytokeratin, EMA (epithelial membran antigen), AFP (alpha fetoprotein) and HCD. The examined lymph nodes showed the same characteristics.

To determine whether the tumour was of donor- or recipient origin DNA typing was performed on the removed tumour and compared with the DNA of the donor. DNA was obtained by trypsin digestion of the tumour tissue followed by the salting-out method. DNA typing was performed by PCR-SSP (sequence specific primer) [22, 23] and the key-reactions were repeated by the more sensitive nested PCR (polymerase chain reaction) technique [24–26]. The result of DNA typing on the basis of HLA mismatches excluded the donor origin of the tumour. We concluded that the tumour represented a de novo malignancy and was not transmitted by the donor (Fig. 5)

The patient received targeted oncological therapy: dacarbazin (200 mg/m^2) for 5 days (with 3 days interruptions) for 1 year. Control ultrasonography showed an 8-cm large recidive mass in the iliac fossa. On control chest X-ray the pulmonary metastases disappeared. Reoperation was performed: a local recurrence was found

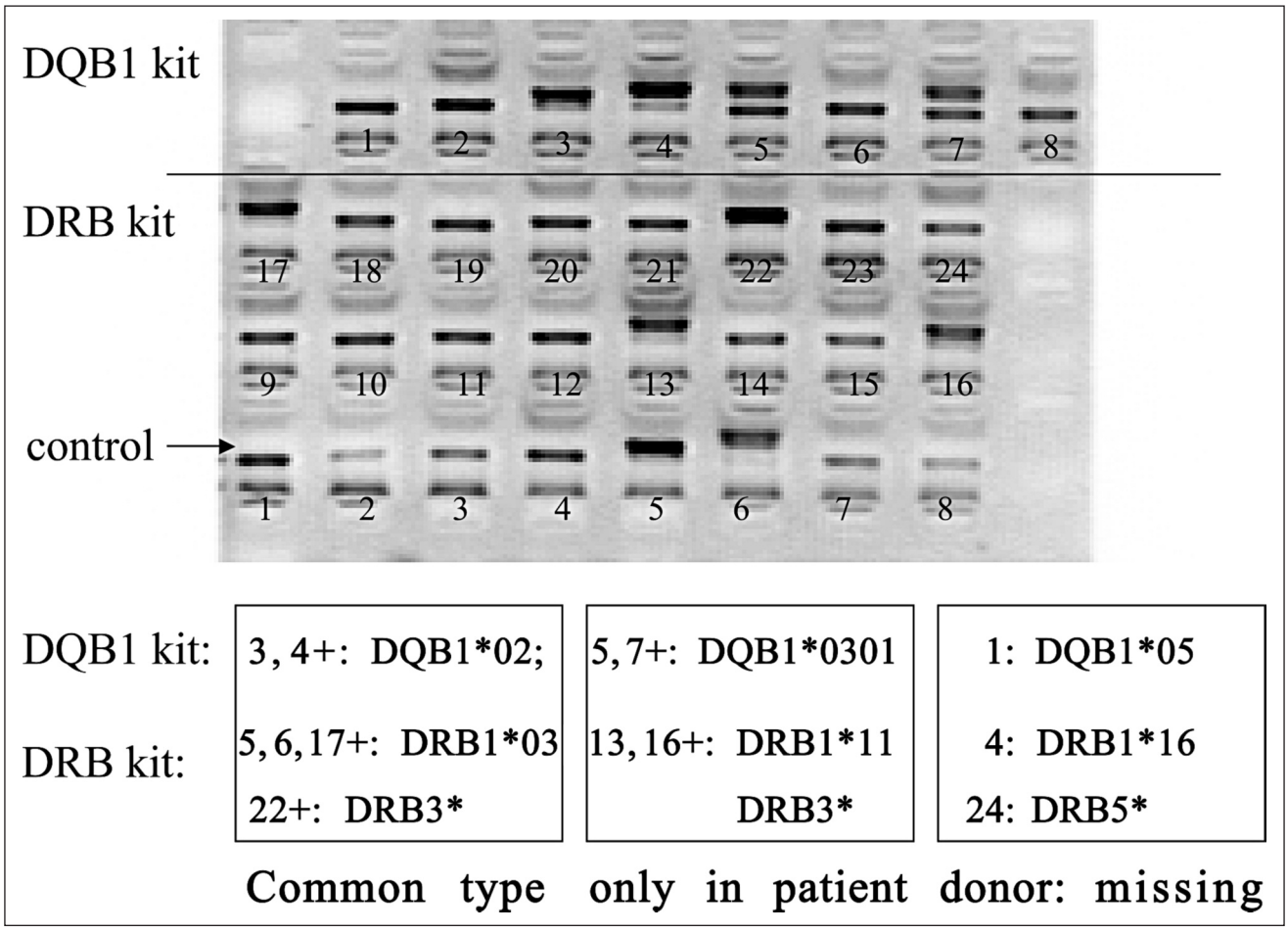

Fig. 5. Result of DNA typing of the tumour tissue. Agarose gel stained by ethidium bromide *First row: results of DQB1 typing*: positive reactions are: 3, 4, 5, 7, which means DQB1*02,03 (patient's type). Reaction 1 (DQB1* characteristic to the donor) is completely missing, even in more sensitive nested PCR (data not shown). *Second row: DRB 1 typing results*: 5, 6, 13, 16, 17, 22, which means DRB1*03,11 and DRB3* (patient's type). Donor specific reactions (4: DRB1*16 and 24: DRB%) are missing. The results of DNA typing confirmed the origin of the tumour

and removed. Histology of the removed tissue and lymph nodes did not reveal signs of malignancy. Reactive changes in the lymph nodes and scar tissue with signs of fibrosis were confirmed. The patient returned to regular haemodialysis treatment, and following 6 (six) tumour-free years after the transplantectomy and 5 (five) years after completing the oncological therapy he was put on the kidney transplant waiting list again. Ten years after transplant nephrectomy he was transplanted with a cadaveric kidney with 1 HLA A, 2 HLA B and 1 HLA DR matches. The donor was CMV positive and the recipient CMV negative, so he received valganciclovir prophylaxis for 3 months. The immunosuppressive therapy consisted of Zenapax, Tacrolimus, Mycophenolate-mofetil and steroids. On the 7th postoperative day clinical signs of rejection appeared, so the patient received steroid pulse therapy for 5 consecutive days. His serum creatinine level decreased.

Because of previous malignant melanoma his immunosuppressive therapy was converted to the combination of Sirolimus, Mycophenolate-mofetil and steroids 2 months after the transplantation. His kidney function is stable, with a serum creatinine 112 μmol/l, GFR: 80 ml/min. The regular screening tests for malignancy did not reveal any abnormalities.

Discussion

Tumour development is an increasingly recognized problem in kidney transplanted patients. The incidence of malignancy is higher in transplanted patients than in the general population. Since the development of immunosuppressive therapy transplanted patients live longer, however, have a greater chance to develop malignant diseases. Length and intensity of immunosuppressive therapy are related to the risk of post-transplant malignancy. The risk of developing malignancy is 14% at 10 years and 40% at 20 years after transplantation.

The leading cause of death in kidney transplant recipients with a functioning graft are cardiovascular complications. Malignant tumours are the second most common cause of death after kidney transplantation [27, 28].

Immunosuppression has been identified as an etiological factor in the increased incidence of malignancies in kidney transplant recipients [28]. Immunosuppression has a non-specific and a direct effect on tumour genesis. Non-specific immunosuppression enhances oncogenic virus replication. Steroids are believed not to be involved in cancer development. Calcineurin inhibitors and azathioprin are relevant risk factors. Azathioprin reduces DNA repair ability. Calcineurin inhibitors are involved through various mechanisms in tumour development: They promote B-cell proliferation by increasing T lymphocyte IL-6 secretion, decrease DNA repair ability. They also might be able to promote the spreading of metastases by direct cellular effect that is independent of their effect on the host's immune cells. Proliferation signal inhibitors are involved in the control of cellular growth and proliferation. Recent articles report that mTOR inhibitors have anticancer effects as well as immunosuppressive properties. In vitro and in vivo studies have documented the ability of proliferation signal inhibitors to inhibit primary and metastatic tumour growth, to stop the cell cycle in G1 phase. They decrease the expression of vascular endothelial growth factor (VEGF-A) and the IGF-β expression.

The incidence of different tumours varies in different geographical regions [29, 30]. Existing studies are not in agreement over the increased risk of melanoma in the transplanted population. Hollenbeak et al. showed that renal transplantation recipients were nearly 3.6 times more likely to develop melanoma than the general population, Le Mire et al. showed that in the studied Oxford transplant population melanomas occurred at approximately 8 times the rate in the general population; this is the highest rate reported in the literature [18, 31].

Considering the well-known aggressivity of melanomas this is also an uncommon finding that after multidisciplinary treatment (surgery, oncology) the patient became tumour and metastasis free. Melanomas transmitted from a donor were already reported [32, 33] and advanced donor origin was proved by DNA typing as well [34, 35]. However, we did not find any report in the literature, where a malignant melanoma occurred after transplantation without any skin lesion and the donor origin was excluded by DNA typing. We highlight the importance of monitoring for de novo tumours in immunosuppressed population, and also the role of DNA typing in the determination of the tumour's origin. In case of multiorgan donation the importance of donor transmitted tumours is even higher. Tumours have uncommon behaviour in immunosuppressed patients. To achieve good prognosis multidisciplinary treatment is suggested.

References

1. Kaufmann HM, Cherikh WS, McBride MA et al.: Deceased donors with past history of malignancy: An organ procurement and transplantation network/united network for organ sharing update. Transplantation 84, 272–274 (2007)
2. Kaufmann HM, Cherikh WS, McBride MA et al.: Post transplant de novo malignancies in renal transplant recipients: The past and the future. Transplant Int 19, 607–620 (2006)
3. Sandrini S: Cancer after kidney transplantation. Giornale Italiano di Nefrologia. 21 Suppl, 26 S67–S73 (2004)
4. Végső G, Tóth M, Hídvégi M et al.: Malignancies after renal transplantation during 33 years at a single center. Pathol Oncol Res 13, 63–69 (2007)
5. Heynemann H, Hamza A, Wagner S et al.: Malignant neoplasms and kidney transplantation. Urologe 48, 1443–1451 (2009)
6. Langer RM, Járay J, Tóth A et al.: De novo tumours after kidney transplantation: The Budapest experience. Transplant Proc 35, 1396–1398 (2003)
7. Laing ME, Moloney FJ, Kay EW et al.: Malignant melanoma in transplant recipients: Review of five cases. Clin Exp Dermatol 5, 662–664 (2006)
8. Wilson LJ, Horvat RT, Tilzer L, Meis AM, Montag L, Huntrakoon M: Identification of donor melanoma in a renal transplant recipient. Diagn Mol Pathol 1, 266–271 (1992)
9. Thaunat O, Morelon E: Cancer and immunosuppression: Pro- and antitumoral effects of immunosuppressive drugs. Nephrol Ther 1, 23–30 (2005)
10. Wimmer CD, Rentsch M, Crispin A et al.: The Janus face of immunosuppression – de novo malignancy after renal transplantation: The experience of the Transplantation Center Munich Kidney Int 12, 1271–1278 (2007)
11. Montegnino G, Lorca E: Cancer incidence in 854 kidney transplant recipients from a single centre. Clin Transplant 10, 461–469 (1996)
12. Penn I: Malignant melanoma in organ allograft recipients. Transplantation 61, 274–278 (1996)
13. Penn I: Primary kidney tumours before and after transplantation. Transplantation 59, 480–485 (1996)
14. Schmidt R, Stippel D: Tumours after renal transplantation. Urol Int. 57, 21–26 (1998)
15. Werner W, Wunderlich B et al.: De novo malignant tumours after renal transplantation. Nieren Hochdruckkr 27, 461–467 (1998)
16. Kim JK, Carmody IC, Cohen AJ et al.: Donor transmission of malignant melanoma to a liver graft recipient: Case report and literature review. Clin Transplant 23, 571–574 (2009)
17. Stephens JK, Everson GT, Elliott CL et al.: Fatal transfer of malignant melanoma from multiorgan donor to four allograft. Transplantation 70, 232–236 (2000)
18. Hollenbeak CS, Todd MM, Billingsley EM, Harper G, Dyer AM, Lengerich EJ: Increased incidence of melanoma in renal transplantation recipients. Cancer 104, 1962–1967 (2005)
19. Vajdic CM, van Leeuwen MT, Webster AC et al.: Cutaneous melanoma is related to immune suppression in kidney transplant recipients. Cancer Epidemiol Biomarkers Prev 18, 2297–2303 (2009)
20. Suranyi MG, Hogan PG, Falk MC et al.: Advanced donor-origin melanoma in a renal transplant recipient: Immunotherapy and retransplantation. Transplantation 66, 655–661 (1998)
21. Mackie RM, Reid R, Junor B et al.: Fatal melanoma transferred in a donated kidney 16 years after melanoma surgery. Letter to the editor. N Eng J Med 348, 567–568 (2003)
22. Bein G, Gläser R, Kirchner H: Rapid HLA-DRB1 genotyping by nested PCR amplification. Tissue Antigens 39, 68–73 (1999)
23. Bunce M, Barnardo MC, Procter J, Marsh SG, Vilches C, Welsh KI: High resolution HLA-C typing by PCR-SSP. Tissue Antigens 50, 100–111 (1997)
24. Keresztury L, Rajczy K, Tauszik T et al.: DNA typing revealing high HLA-Cw polymorphism completes availability of major histocompatibility complex loci in forensic medicine. Am J Forensic Med Pathol 24, 70–75 (2003)
25. Miller SA, Dykes DD, Polesky HF et al.: A simple salting procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16, 1215–1218 (1988)

26. Schmitt C, Ciré K, Schattenkirchner S et al.: Highly sensitive DNA typing for detecting tumors transmitted by transplantation. Transpl. Int 11, 382–386 (1998)
27. Kasiske BL, Snyder JJ, Gilbertson DT, Wang C: Cancer after kidney transplantation in the United States. Am J Transplant 4, 905–913 (2004)
28. Kaufmann HM, Cherikh WS, Cheng Y et al.: Maintenance immunosuppression with target of rapamycin inhibitors is associated with a reduced incidence of de novo malignancies. Transplantation 80, 883–889 (2005)
29. Nemes B, Podder H, Járay J et al.: Primary hepatic carcinoid in a renal transplant patient. Pathol Oncol Res. 5, 67–69 (1999)
30. Nemes B, Zalatnai A, Podder H et al.: Papillary microcarcinoma of the thyroid gland in renal transplant patients. Pathol Oncol Res 6, 72–75 (2000)
31. Le Mire L, Hollowood K, Gray D, Bordea C, Wojnarowska F et al.: Melanomas in renal transplant recipients. Br J Dermatol 154, 472–477 (2006)
32. Milton CA, Barbara J, Cooper J et al.: The transmission of donor-derived malignant melanoma to a renal allograft recipient. Clin Transplant 20, 547–550 (2006)
33. Morris-Stiff G, Steel A, Savage P et al.: Transmission of donor melanoma to multiple organ transplant recipients. Am J Transplant 4, 444–446 (2004)
34. Olerup O, Zetterquist H: HLA DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. Tissue Antigens 39, 225–235 (1992)
35. Tissue Typing DYNAL-SSP Technical Handbook. 1st Edition. 29, 52–53 (1996)