Aggressive melanoma in an infant with congenital melanocytic nevus syndrome and multiple, *NRAS*- and *BRAF*-mutation negative nodules

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Number of words, excluding abstract and references: 1389

Abstract

We report the case of a newborn boy with multinodular *NRAS*- and *BRAF*-mutation negative congenital melanocytic nevi and cerebral lesions compatible with congenital intraparenchymal melanosis. Histopathology from skin lesions showed atypical nodular melanocytic proliferation with marked melanocytic atypia and a high number of mitoses and apoptosis, indicating aggressive proliferation. The child developed several new subcutaneous tumors and multiple internal lesions, which were confirmed to be metastases, and died five months old. This case may represent an infantile melanoma developing from a giant congenital melanocytic nevus, or a congenital melanoma.

Number of words: 89

Congenital melanocytic nevi (CMNs) may vary greatly at birth, from single small macules to large nodules with multiple satellite lesions covering most of the body surface with or without additional involvement of the central nervous system.[1] Although most CMNs do not change significantly, some may develop changes of color or nodules in a short period of time, making clinical and histological findings difficult to interpret. We present a case that highlights the challenges involved in assessing such patients.

Case report

A 32-year-old pregnant woman was transferred to our hospital following a 3D ultrasound at week 27 of gestation, with findings suggesting a vascular malformation in the fetus. A previous routine pregnancy ultrasound at week 20 was normal. Both parents were healthy and unrelated with no known hereditary diseases, and they had a 3-year old healthy daughter.

A boy, weighing 4 kg with a length of 51cm was delivered by caesarian section at 37 weeks. Apgar scores were 6 and 7 at 1 and 5 minutes, respectively. The boy had a large, multilobular, pigmented lesion covering both the anterior and posterior aspects of the lower body, including buttocks and genital area (Figure 1), as well as multiple pigmented macules and nodules on the extremities, head and face. The total body surface area covered by pigmented lesions was approximately 35%. The largest lesion on the lower part of the torso had a partially eroded surface and a spongy, non-pitting consistency, which diminished considerably within two days, and was consequently perceived as declining edema. The placenta had a macroscopic normal appearance, with a size of 17.5x18cm and a net weight of 778 g.

A thoraco-abdominal magnetic resonance imaging scan (MRI) one day after birth showed that the largest skin lesion infiltrated the subcutaneous fat, with no deeper extension or additional

abnormalities in the chest, abdomen or extremities (Figure 2a). There was high signal in T2 Turbo Spin Echo (TSE) Dixon sequence in the dermal layer of the lower torso, corresponding to the largest lesion, compatible with edema. A craniospinal MRI scan showed several foci with high signal in T1 in both parenchyma and cortex of both hemispheres. These results were compatible with intraparenchymal melanosis and confirmed a diagnosis of congenital melanocytic nevus syndrome.[1] The infant was otherwise in good health and seemingly unaffected by the skin and brain lesions.

In total, 14 biopsies were taken for histopathological examination. A biopsy from the background giant nevus showed benign compound nevus cell tumor with hyperproliferation and melanophages. Biopsy from the largest pedunculated nodule showed atypical nodular melanocytic proliferation with marked nuclear atypia, an increased number of mitoses (13 mitoses/mm^2), but no necrosis. Areas with neuroid differentiation with neurofibromatous tissue and vascular malformation, consistent with heterologous tissue were also identified (Figure 3). Biopsies from two pedunculated nodules on the right side of the abdomen and hip, and three nodules on the abdomen, right thigh, and the right groin had the same histopathological pattern of atypical melanocytic proliferation with an increased number of mitoses, consistent with atypical nodular melanocytic proliferations.[2] Initial reports had mitotic figures as low as 6/mm^2 without apoptosis or necrosis. Subsequent reports had increasing mitotic figures of up to 25/mm^2 and additional apoptosis or necrosis. Biopsies of two erythematous papules on the torso had histopathological findings consistent with spindle-cell dermal benign nevus-cell tumor. The remaining samples were biopsies from lesions on the torso and scalp with histopathological findings consistent with benign nevi. There was expression of known melanocytic markers, such as S-100, HMB45 and Melan A, in tumor cells. *BRAF* codon 600 and *NRAS* codon 61 mutations

were not detected in any biopsies from any of the many lesions. These negative results were confirmed by preliminary analysis of deep next-generation sequencing. Histopathological analysis of the placenta showed dissemination of melanocytes in some of the villi (Supplemental figure 1). Increased proliferative capacity was indicated by Melan A and Ki-67 staining, but the cells were not perceived as atypical by the pathologist.

Our working diagnosis was *NRAS*- and *BRAF*-mutation negative CMN syndrome with a prominent multinodular phenotype,[3] classified as a giant CMN (G2) of trunk, S3, C2, R2, N2, H0.[4] Surgery was performed to excise those tumors that hindered nursing care or were thought to cause discomfort. Surgery was deemed necessary on three occasions; the first time shortly after birth in order to remove a 7 cm large partially eroded nodular part of the main lesion on the right side of the abdomen that was slightly pedunculated, thus hindering proper hygiene and diaper use. A second operation was performed one month later in order to alleviate relapsing intertrigo. Two months later, a rapidly growing tumor in the right groin causing a secondary hydrocele was removed (Figure 2b). This tumor was biopsied and histopathological examination showed atypical nodular melanocytic proliferation with apoptosis and mitotic figures of 10/mm^2. The lesion was consequently perceived as atypical nodular melanocytic proliferation and was removed one week later. Histopathologic examination of the excised tumor showed the same findings as in the previous biopsy, but with increased mitotic figures of 25/mm^2.

One month later, the patient had developed a relapsing groin tumor and secondary hydrocele, as well as a subcutaneous tumor in the right temporal area and a smaller one in the left temporal area, neither of which were covered by a nevoid lesion (Figure 4). An ultrasound revealed a tumor located right above the periosteum of the temporal bone on each side, with a maximum extracranial diameter of 2.8 cm on the right, and with additional bone destruction and

intracranial invasion on the left (Figure 5). A thoraco-abdominal MRI revealed multiple new non-enhancing T2-intense lesions in the liver, compatible with necrosis (Figure 2c). Fine needle aspiration cytology from both temple tumors and from the right groin tumor showed atypical melanocytic proliferation with enlarged nuclei, distinct nucleoli, and necrosis. All three tumors showed HMB45 expression.

A diagnosis of metastatic melanoma, primary site unknown, was made. Palliative care was agreed upon, consisting of pain relief and parenteral hydration. The patient died three weeks later.

Discussion

Hypercellularity, atypia and increased mitotic figures are all clear signs of malignancy. However, these were simultaneously found in several nodules from different areas of the skin in the neonatal period. Thus, these were perceived as atypical nodular melanocytic proliferations. There are currently no reliable markers that can discern between proliferative nodules and melanoma in patients with CMN during infancy, although copy number analysis and markers of methylation may be helpful.[5, 6] Tissue and blood samples are currently been analyzed by whole-genome sequencing and array CGH as part of an on-going, multi-patient study on CMN.

A main challenge in this unusual case was the extent and depth of nodular disease at birth, rendering active surgical management unlikely to alter outcome even if a diagnosis of melanoma could have been made. In addition, kinase inhibition treatment was not an option due to the *NRAS*- and *BRAF*-negative status of the lesions. Other published cases of CMN syndrome have been shown to be caused by post-zygotic *NRAS*-mutations in approximately 80%.[7, 8] We did not, however, find any *NRAS* mutations (codons 12, 13, 61) or *BRAF* mutations. Checkpoint

inhibition therapy was not considered to be an option due to the rapid and fatal course of the case.

The risk of developing melanoma from CMNs varies with congenital phenotype; a recent review and prospective cohort study concludes that the average melanoma incidence for all CMNs is 1-2%, with wide variation depending on the severity of the congenital phenotype. For large CMNs with satellite lesions, the lifetime risk rises to 10-15%.[9-11] The strongest risk factor appears to be abnormal MRI screening of the CNS in the first months of life, with a melanoma incidence of 12%.[9, 12, 13]

The literature does not support surgery as a prophylactic intervention to avoid malignant transformation.[12, 14] Consequently, management of large CMN and the potential need for surgery should be individualized according to melanoma risk, as well as aesthetic and psychosocial concerns. It is unclear whether our patient had a congenital melanoma or a giant CMN with malignant transformation. The case illustrates the difficult diagnostic, therapeutic and management approaches in such patients.

Acknowledgements

We thank Petter Gjersvik, M.D., Ph.D., for editorial support, and the Medical Photography and Illustration Service at the University of Oslo.

Written informed consent for publication of this case report has been obtained from both parents.

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Legends to figures:

Figure 1: Clinical appearance right after birth.

Figure 2: Thoraco-abdominal MRI showing status at birth (a), at 3 months of age, with inguinal tumor (b, white arrow), and at 5 months of age, with relapsing inguinal tumor and liver metastases (c, white arrows).

Figure 3: Histopathologic section of the largest nodule removed on the first operation, showing proliferation of atypical melanocytes, with frequent mitoses (red arrows) and apoptosis (yellow arrows) (Hematoxylin and Eosin 400X).

Figure 4: Clinical appearance of subcutaneous tumors in each temporal area which developed during the third month of age.

Figure 5: Ultrasound image from the left temporal area showing a tumor measuring 1.55 x 0.77 cm with bone destruction.

Supplemental figure 1: Histopathologic section of placental villi showing disseminated proliferation of melanocytes, highlighted with labelled antibodies against Melan A and Ki-67 (Hematoxylin and Eosin 400X).