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# History of chronic inflammatory disorders increases the risk of Merkel cell carcinoma, but does not correlate with Merkel cell polyomavirus infection

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**Background:** We aimed to assess the connection between chronic inflammatory disorders (CIDs) and Merkel cell carcinoma (MCC).

**Methods:** Merkel cell carcinoma cases diagnosed in 1978–2009 were extracted from the Finnish Cancer Registry and controls from the Population Registry. Information on reimbursed CIDs was linked to clinicopathological data including Merkel cell polyomavirus (MCV) status by qPCR and immunohistochemistry for the large T antigen of MCV (LTA), Ki-67 and tumour-infiltrating lymphocytes.

**Results:** Chronic inflammatory disorders increased the risk of MCC significantly (odds ratio (OR) 1.39, 95% confidence interval (CI) 1.03–1.88), specifically connective tissue/systemic diseases (OR 1.75, 95% CI 1.09–1.80) and diabetic conditions (OR 1.51, 95% CI 1.03–2.22). Chronic inflammatory disorders associated with larger tumour diameter ($P=0.02$) and higher Ki-67 expression ($P=0.005$). The expression of LTA was seen significantly more often in the absence of CIDs ($P=0.05$).

**Conclusions:** Patients with CID are at significantly higher risk for aggressive MCC. Merkel cell polyomavirus positivity is more common in MCC patients unafflicted by CID.

Merkel cell polyomavirus (MCV) has a causal role in the tumourigenesis of Merkel cell carcinoma (MCC) (WHO, 2013). Recent evidence implicates two subgroups of MCC tumours: the MCV-positive and MCV-negative group, which differ in their clinical characteristics and pathobiological properties (Leroux-Kozal *et al*, 2014; Sahi *et al*, 2014; Veija *et al*, 2015). Merkel cell polyomavirus positivity of the tumour is associated with a favourable prognosis. However, the prognosis of MCV-positive patients is dependent on the intensity of antiviral immune response (Paulson *et al*, 2011), and immunosuppression leads to poorer outcome regardless of MCV status (Paulson *et al*, 2013). Higher numbers of tumour invading CD8+ lymphocytes in MCV-positive tumours correlates with a favourable MCC prognosis (Paulson *et al*, 2011; Sihto *et al*, 2012; Harms *et al*, 2013).

The association between chronic inflammatory disorders (CIDs) and MCC has received less systematic attention than other immunocompromising states such as HIV infection or post-transplant immunosuppression (Arron *et al*, 2014). We sought to examine the risk for MCC in patients with CIDs in a nationwide case–control setting. Further, we linked the epidemiological data to the clinicopathological data of the MCC cases to assess whether CIDs correlate with the course of the disease or the tumours' clinical characteristics.

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## MATERIALS AND METHODS

In phase I, registry data were used in an epidemiological case–control setting to determine whether CIDs affect the risk for MCC. In phase II, MCC patients included in phase I were linked via their personal identity codes to their clinicopathological characteristics.

An Institutional Ethics Committee approved the study plan. The National Institute of Health and Welfare granted its permission to use the information of the Finnish Cancer Registry (FCR). The National Social Insurance institution (SII) provided permission to access drug reimbursement data.

Our group has gathered all paraffinised primary MCC tumour samples available in Finland since 1978. During the past 10 years, we have compiled a database of information on the patient characteristics and course of the disease, as well as tumour characteristics including viral status, tumour genomics and the expression of various immunohistochemical markers. The ongoing

**Table 1. SII categories with the chronic inflammatory disorders included in the study**

| SII category | Chronic inflammatory disorder | ICD-10 |
|---|---|---|
| **Systemic/connective tissue** | | |
| Rheumatic diseases and conditions | Enteritis due to *Yersinia enterocolitica* | A04.6 |
| | Other meningococcal infections | A39.8 |
| | Other late congenital syphilis, symptomatic | A50.5 |
| | Langerhans cell histiocytosis not elsewhere classified | D76.0 |
| | Other histiocytosis syndromes | D76.3 |
| | Chronic iridocyclitis | H20.1 |
| | Chorioretinal inflammation | H30 |
| | Acute and subacute infective endocarditis | I33.0 |
| | Other interstitial pulmonary diseases | J84 |
| | Crohn's disease, unspecified | K50.9 |
| | Ulcerative colitis, unspecified | K51.9 |
| | Chronic active hepatitis, not elsewhere classified | K73.2 |
| | Autoimmune hepatitis | K75.4 |
| | Primary biliary cirrhosis | K74.3 |
| | Cholangitis | K83.0 |
| | Arthropathic psoriasis | L40.5 |
| | Postinfective and reactive arthropathies | M02 |
| | Rheumatoid arthritis with rheumatoid factor | M05 |
| | Other rheumatoid arthritis | M06 |
| | Juvenile arthritis | M08 |
| | Arthritis, unspecified | M13.9 |
| | Polyarteritis nodosa and related conditions | M30 |
| | Other necrotising vasculopathies | M31 |
| | Systemic lupus erythematosus (SLE) | M32 |
| | Dermatopolymyositis | M33 |
| | Systemic sclerosis | M34 |
| | Other systemic involvement of connective tissue | M35 |
| | Ankylosing spondylitis | M45 |
| | Sacroiliitis, not elsewhere classified | M46.1 |
| | Unspecified inflammatory spondylopathy | M46.9 |
| | Relapsing polychondritis | M94.1 |
| | Chronic nephritic syndrome | N03 |
| | Atresia of bile ducts | Q44.2 |
| Sarcoidosis | Sarcoidosis | D86 |
| Ablastic anaemia | Acquired pure red cell aplasia | D60 |
| | Other aplastic anemias and other bone marrow failure syndrome | D61 |
| **Gastrointestinal** | | |
| Colitis ulcerosa, Morbus Crohn | Morbus Chron | K50 |
| | Colitis ulcerosa | K51 |
| **Skin** | | |
| Pemphigus | Pemphigus | L10 |
| | Pemphigoid | L12 |
| General erythrodermia | Lichen planus | L43 |
| | Psoriasis | L40 |
| | Systemic lupus erythematosus | M32 |
| **Neural system** | | |
| Parkinson's disease, comparable disorders | Parkinson's disease | G20 |
| Multiple sclerosis | Multiple sclerosis | G35 |
| Myasthenia gravis | Myasthenia gravis | G70.0 |
| **Diabetic conditions** | | |
| Diabetes mellitus | Type 1 diabetes mellitus | E10 |
| | Type 2 diabetes mellitus | E11 |
| | Diabetes due to malnutrition | E12 |
| | Other specified diabetes mellitus | E13 |
| | Unspecified diabetes | E14 |
| | Postprocedural hypoinsulinemia | E89.1 |

Abbreviation: SII = social insurance institution. The categories are further grouped according to the affected organ.

MCC projects of our research group continue to utilise this database.

**Phase I.** Merkel cell carcinoma cases diagnosed in 1978–2009 were identified from the FCR. They were matched for 15 referent persons randomly selected from the Population Register Centre based on the following criteria: (1) same gender and (2) same year of birth. Of these 15 persons, those alive at the time of the MCC diagnosis of the case served as controls.

The SII manages a database that includes specific data on medicine expense reimbursements for chronic diseases. We retrieved data on the SII categories comprising CIDs (Table 1) by using the personal identity codes of the cases and controls as a key. Only diseases prior to MCC diagnosis date were noted.

On the basis of statistical power analysis, the CIDs were grouped together for further calculations according to the affected organ systems (Table 2). A conditional logistic regression model was used to estimate, by means of the odds ratio (OR) with 95 % confidence intervals (95% CI), the risk for MCC.

**Phase II.** Merkel cell carcinoma patients identified in phase I were linked via personal identity codes to the clinicopathological data of our MCC patient–tumour database. The clinical data comprised the sex of the patients, the pattern of metastatic dissemination and tumour recurrence rates. We examined metastasis in two alternative categories, with a division between local and metastasised disease or local disease, nodal metastasis and systemic metastasis. Combined with immunohistochemistry for Ki-67, these characteristics were used to chart how aggressive the disease was.

The real-time qPCR method used to quantify MCV DNA appears in detail elsewhere (Sihto *et al*, 2012). We also retrieved previously gathered data on the expression of the large T antigen of MCV (LTA) and Ki-67 for the cases (Sihto *et al*, 2012). Ki-67 staining was classified into three categories: low (0–32.7% positively staining tumour cell nuclei, 35 tumours), medium (33.6–66% positive nuclei, 36 tumours) and high (61–100% positive nuclei, 36 tumours). We also analysed Ki-67 expression as a continuous variable. The absolute numbers of tumour-infiltrating lymphocytes (CD68+, CD8+ and CD3+) were detected by scanning the whole-tumour sections after histochemical labelling (Sihto *et al*, 2012) and divided into tertiles for the statistical analysis (Supplementary Table 1).

Pearson's $\chi^2$-test was used for the analysis of categorical variables, and the Mann–Whitney *U*-test for continuous variables. We used Kaplan–Meier analysis, log-rank test and Wilcoxon test to analyse differences in overall survival between the subgroups defined by inflammatory history and Ki-67 expression (high tertile *vs* low and

**Table 2. The numbers (N) of CID in cases with MCC and controls, as well as OR and their 95% CI**

| | *N* MCC | *N* Controls | OR | 95% CI |
|---|---|---|---|---|
| Any chronic inflammatory disorder or multiple[a] | 59 | 487 | 1.63 | 1.19–2.22 |
| **Chronic inflammatory disorders by anatomical involvement[b]** | | | | |
| Systemic/connective tissue diseases | 23 | 144 | 2.01 | 1.26–3.20 |
| Rheumatic diseases and conditions | 22 | 140 | 1.96 | 1.22–3.15 |
| Sarcoidosis | 1 | 4 | | |
| Ablastic anaemia | 0 | 1 | | |
| Dermatologic diseases | 1 | 3 | | |
| Erythrodermia | 0 | 2 | | |
| Pemphigus | 1 | 1 | | |
| Gastrointestinal diseases | 1 | 8 | 1.59 | 0.20–12.8 |
| Neural diseases | 5 | 76 | 0.75 | 0.29–1.91 |
| Parkinsons disease | 5 | 72 | 0.78 | 0.30–2.00 |
| Multiple sclerosis | 0 | 4 | | |
| Myasthenia gravis | 0 | 1 | | |
| Diabetic conditions | 35 | 296 | 1.51 | 1.03–2.22 |

Abbreviations: CI = confidence interval; CID = chronic inflammatory diseases; MCC = Merkel cell carcinoma; OR = odds ratios.
[a]List of all CID included in the study is given in Table 1.
[b]As defined in Table 1.

**Table 3. Numbers of MCC tumours according to variables depicting the viral status and malignant tendency of the disease, and by the history of chronic inflammatory disease**

| | Choric inflammatory disorder(s) | No chronic inflammatory disorders | *P*-value | |
|---|---|---|---|---|
| **Viral status** | | | | Total number of tumours |
| MCV PCR+(%) | 23/36 (64) | 69/90 (77) | 0.14 | 126 |
| MCV LTA+(%) | 19/36 (53) | 60/84 (71) | 0.0048 | 120 |
| Median MCV copy number, per ref. gene (range) | 0.89 (0–4224) | 1.07 (0–2968) | 0.50 | 126 |
| **Clinical behaviour** | | | | Total number of patients |
| Local, nodal or systemic metastasis (%) | 9 /42 (21) | 44/139 (32) | 0.360 | 181 |
| Mean tumour size, mm (range) | 21 (4–70) | 17 (3–85) | 0.025 | 134 |

Abbreviations: MCC = Merkel cell carcinoma; MCV = Merkel cell polyomavirus; LTA = large T antigen of MCV. The clinical data and tumour material available for each analysis varied, with the total number of assessed patients/tumours for each variable listed in the last column. When evaluating the statistical significance in the variables between the two patient subgroups, *P*-values (*P*) < 0.05 were considered significant.

medium tertiles). Multivariate survival analysis was conducted with the Cox regression model. The variables consisted of age, gender, history of CID, tumour size, LTA expression and Ki-67 expression.

## RESULTS

**Phase I: chronic inflammatory disorders and the risk of MCC.** The final case–control study set comprised 267 cases and 3 270 controls. Of the 267 MCC patients, 59 (22.1%) were diagnosed with at least one CID, whereas the respective number for the controls was 487 (14.9%). Chronic inflammatory disorders led to significantly higher risk for MCC (OR 1.63, 95% CI 1.19–2.22). The OR was not significantly affected by the gender or age of the MCC patients.

The history of inflammatory systemic/connective tissue disorders significantly increased the risk for MCC (OR 2.01, 95% CI 1.26–3.20), due mainly to rheumatoid conditions, which had an OR of 1.96 (95% CI 1.22–3.15). In addition, diabetic conditions significantly increased the risk for MCC (OR 1.51, 95% CI 1.03–2.22). Neural and gastrointestinal CIDs were rare. The results appear in full in Table 2.

**Phase II: chronic inflammatory disorders and features of MCC tumours.** Data on MCV status were available for 142 MCC patients (Supplementary Figure 1), on Ki-67 expression for 107 patients, and on the TILs for 129 patients on the CD68+ stain, 93 on the CD8+ stain and 128 for the CD3+ stain. Information on tumour size and metastasis was available for 134 patients.

Merkel cell polyomavirus DNA was more frequently found in MCC tumours of patients with no CIDs (77% *vs* 64%, Table 3), although the finding was not statistically significant. The LTA, however, was expressed significantly more often in the absence of CID (71% *vs* 53%, $P=0.05$). We observed no significant difference in the viral copy numbers between the groups (Table 3).

The expression of Ki-67 was significantly higher in tumours of MCC patients with CID, with a mean percentage of 63% *vs* 56% ($P=0.005$; Supplementary Table 1). The finding was further strengthened by tertile analysis ($P=0.007$). Chronic inflammatory disorders associated with a larger tumour diameter $P=0.02$ (Table 3), but the statistical significance disappeared when the tumours were stratified by MCV status ($p^{MCV+}=0.06$, $p^{MCV-}=0.23$). Chronic inflammatory disorders did not associate with progression to metastatic disease (Table 3). No significant differences were detected in the overall survival or the TIL numbers between the subgroups (Supplementary Table 1). In the multivariate survival analysis, the only statistically significant variable was age at MCC diagnosis (hazard ratio = 1.033, 95% CI 1.002–1.066, $P=0.036$).

## DISCUSSION

We identified a statistically significant increase in the risk for MCC in patients with a history of CIDs. Patients with inflammatory connective tissue diseases and diabetes were at the highest risk for MCC. The results are similar to the relative risk estimates of MCC following rheumatoid arthritis published in the United States (OR 1.39, 95% CI 1.10–1.74) and Sweden (SIR 2.42, 95% CI 0.96–5.01) (Lanoy and Engels, 2010; Hemminki *et al*, 2012). The tumours of patients with a history of CID were also larger and expressed elevated levels of the proliferation marker Ki-67. This supports the earlier notion of the aggressive course of the disease in immunodefective individuals (Paulson *et al*, 2013).

Merkel cell carcinoma patients diagnosed with CIDs presented frequently with MCV qPCR-positive tumours. Expression of the LTA occurred in the tumours significantly more often in the absence of CID. The finding is surprising and differs from reports of MCV incidence among the cohort of immunosuppressed MCC patients with chronic lymphocytic leukaemia (Koljonen *et al*, 2009). This finding seems to stress the difference between stark immunosuppression and more subtle changes in the immune responses in patients with chronic inflammation. One could hypothesise that the immunomodulatory role of chronic inflammation in MCC pathogenesis and predisposition to oncogenesis is more complicated, and may involve tumour surveillance instead of surveillance against virus infection. However, no statistically significant difference was detected in the MCV DNA status between the groups, even though it usually correlates accurately with LTA expression. Confirmation of the finding in other MCC cohorts would be informative.

Tumour-infiltrating lymphocytes are under vigorous scrutiny in MCC due to the new immunomodulating treatment options derived from melanoma research. High CD8+/CD4+ and FoxP3+/CD4+ ratios, as well as CD8+ and CD3+ numbers are associated with good survival (Sihto *et al*, 2012). This is the first study to look into the TIL spectrum of MCC tumours in immunocompromised patients. We found no significant differences in the numbers of TILs between the patient groups implicating that immunodeficiencies affect the function rather than the trafficking of TILs into MCC tumours.

The results of this study recommend consideration for MCC, especially when dealing with patients with rheumatic and diabetic diseases, as it is more common and more aggressive in these patients. Further studies are needed to elucidate the specific mechanism behind the effect and the interaction between MCV infection and chronic inflammation.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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