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Merkel cell carcinoma in Taiwan: a subset is chronic arsenicism-related, and the Merkel cell polyomavirus-negative cases are pathologically distinct from virus-related cases with a poorer outcome

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ABSTRACT

Merkel cell carcinoma (MCC) is a rare primary neuroendocrine carcinoma of the skin, more frequent in the West than in the East. The pathogenesis is complex, with Merkel cell polyomavirus (MCPyV) and ultraviolet (UV) light being reported to play important roles. In this retrospective study of MCC cases from Taiwan, we analysed the prognostic significance of pathological features and the status of MCPyV and retinoblastoma (Rb) expression. We retrospectively collected MCC cases from five hospitals in Taiwan from 1994 to 2022. We examined the clinical and pathological features, performed immunohistochemical studies for the large T antigen of MCPyV and Rb, and reviewed medical records from electronic data. Disease-specific survival was estimated by using the Kaplan–Meier estimate and compared between subgroups using log-rank test. The clinical and pathological features and the immunohistochemical profiles between subgroups were compared using the Fisher exact test for categorical variables. The 38 patients were mostly (71%) males, with a median age of 79. In 22 (58%) patients, the tumours occurred in sun-exposed areas. Clinically, five (13%) patients had chronic arsenicism. Histopathologically, 11 (29%) cases showed combined tumours (MCC with squamous cell carcinoma or Bowen disease/squamous carcinoma *in situ*). Seventeen (45%) cases were positive for MCPyV, whereas all combined tumours were negative. MCPyV-negative MCC displayed distinctive pathological features, including epidermal changes, presence of an intraepidermal MCC component, linear or single-file growth pattern, and pleomorphic nuclei. Immunohistochemically, 59% (22/37) MCC cases showed complete loss of Rb protein expression, more frequent in MCPyV-unrelated ($p<0.001$) and combined ($p=0.014$) cases, but without statistical significance among patients with chronic arsenicism, sun exposure, or disease-specific survival. MCPyV-negative cases exhibited a shorter disease-specific survival than MCPyV-positive cases (median overall survival 13 months vs not reached; $p=0.041$). MCPyV-negative or combined MCCs were associated with a higher disease-specific mortality and poorer prognosis. MCCs occurring in sun-shielded sites, MCPyV-negativity, and combined tumours correlated with a higher disease-specific mortality and a poorer prognosis by multivariable Cox proportional hazard model. The occurrence of MCCs with arsenic exposure was also identified. Our study showed that MCPyV-negative MCC cases in Taiwan exhibited distinctive pathological features and a poorer outcome than MCPyV-related cases. We also confirmed an association of chronic arsenicism with MCC, which might be considered as the third pathogenetic

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factor after MCPyV and UV light. Further studies including epidemiological and genetic investigations are warranted to elucidate the pathogenesis of MCC in Taiwan, particularly the significance of chronic arsenicism.

1. Introduction

Merkel cell carcinoma (MCC) is an aggressive skin cancer mainly affecting the elderly and is characterised by both epithelial and neuroendocrine (NE) features.[1–3] MCC most frequently occurs in the sun-exposed areas of the head and neck, followed by the extremities.[2–4] The well-documented risk factors include chronic sun exposure, ultraviolet (UV) A photochemotherapy, white skin type, male sex, immunosuppression, and a history of previous skin malignancies.[2,3] The prevalence of MCC seems to be steadily increasing in most regions of Western countries.[3,5] The incidence varies across different geographical regions, with a relatively low frequency in Asian countries.[6–8] In Taiwan, the annual incidence of MCC was 0.05 per 100,000 individuals in 2020 (Dr Chun-Ju Chiang at National Taiwan Cancer Registry; personal communication), which is relatively low compared to the United States, Europe, and Australia (0.1–1.6 per 100,000).[9]

The pathogenesis of MCC is complex. The two major factors are the integration of the Merkel cell polyomavirus (MCPyV) genome into the host cells and the genetic damage from long-term UV light exposure.[3,9–11] The viral large T antigen of MCPyV plays a critical role in tumorigenesis by binding to and inactivating key tumour suppressor proteins, including the retinoblastoma (Rb) protein. The inactivation of *RB1* disrupts cell cycle regulation, leading to uncontrolled cell proliferation, essential for the development of MCC.[12] Currently, the understanding of MCC primarily comes from retrospective studies conducted in the West. A recently published large cohort study based on 18 population-based cancer registries of the Surveillance, Epidemiology, and End Results (SEER) Program revealed differences in terms of disease characteristics and outcome among different racial/ethnic groups (Asian American or Pacific Islander, Black, Hispanic vs White).[13] Due to the rarity of MCC in Asia, the specific features of this disease and the prognostic factors are largely unknown.

The difference in the prevalence of MCC between Asian and Western populations suggests that both environmental and genetic variables might exert a role. In 1990s, early reports linked MCC with blackfoot disease and chronic arsenicism in Taiwan.[14,15] In our recent study of 18 cases, we found that MCPyV-positive cases had a better survival.[16] In the current study, we expanded our study to 38 patients and analysed the prognostic significance of clinicopathological features and the status of MCPyV and Rb expression.

2. Methods

2.1. Patients and samples

MCC cases were retrospectively collected from five hospitals in Taiwan between 1994 and 2022. Only cases with sufficient formalin-fixed paraffin-embedded tissues were included. The diagnostic criteria included pathologically confirmed primary cutaneous NE carcinoma with a dual immunohistochemical expression of one epithelial marker (such as CK20 in a dot-like pattern) and at least one NE marker, and imaging studies excluding metastasis from the lungs. Diagnoses were confirmed by the contributing pathologists and the coauthors (C-YL, Y-RL and S-SC). The study was conducted according to the Declaration of Helsinki and was approved by the institutional review board at both Chi-Mei Medical Center and Cathay General Hospital.

2.2. Clinical and follow-up data

We gathered the following clinical information including age, sex, features of chronic arsenicism, site and size of MCC, clinical course, treatment, and follow-up information from electronic medical records. We defined MCC patients with underlying chronic arsenicism by the presence of the following distinctive skin lesions including erythematous to pigmented crusted plaques (Bowen disease), punctate keratotic papules on the palms and soles, and a freckled raindrop pattern characterised by hyperpigmented and hypopigmented macules on the trunk. We used the eighth edition of the American Joint Committee on Cancer (AJCC) staging manual for staging.[17] The locations of MCCs were classified into sun-exposed sites (the face, neck, scalp, arm, forearm, wrist, dorsal hand, lower leg, ankle, and dorsal foot) versus sun-protected or sun-shielded sites (the chest, abdomen, back, buttock, thigh, palm, and sole). Case no. 1–10, 12–19, and 21 were previously reported.[16,18]

2.3. Histopathological analysis

Two authors (C-YL and Y-RL) evaluated the histopathological features. The growth pattern, based on the tumour borders under low-power examination, was classified as circumscribed (with pushing borders), infiltrative (with strands, cords, trabeculae, and single cells infiltrating dermal collagen), or mixed (Fig. 1). We also evaluated the presence or absence of intraepidermal MCC component, linear growth pattern (strands or cords of tumour cells), crush artifact, lymphovascular invasion, perineural invasion, tumour-infiltrating lymphocytes, solar elastosis, ulcer, and necrosis (Fig. 1 and 2). Under high-power examination, the tumour cells were classified with the following parameters: size of predominant tumour cells (small cell, <2 lymphocytic nuclei vs large cell, >3 lymphocytic nuclei), nuclear shape (regular/round, elongated, pleomorphic, or mixed), and cytoplasmic volume (none/inconspicuous vs intermediate to abundant). Combined (as opposed to pure) MCCs were defined as those with coexisting squamous cell carcinoma (SCC) or Bowen disease/squamous carcinoma *in situ* (SCIS; Fig. 2).[19–21] We also evaluated the presence or absence of chronic arsenicism-associated epidermal alternations (hyperkeratosis, parakeratosis, papillomatosis, elongation of rete ridges, squamous cell dysplasia, and basal pigmentation; Fig. 3).[22,23]

2.4. Immunohistochemistry

We used 4-μm-thick paraffin sections and a polymer-based detection system for immunohistochemistry in Leica Bond-MAX autostainer (BOND-III, Leica BioSystems). The antibodies used included cytokeratin (CK) AE1/AE3, CK20, CD56, synaptophysin, chromogranin A, thyroid transcription factor-1 (TTF-1, clone SP141), insulinoma-associated protein 1 (INSM1), MCPyV large T antigen (clone CM2B4, Santa Cruz), and Rb (clone 13A10, Novocastra). MCPyV status was classified according to the percentage of tumour cells showing positive nuclear staining as either positive ($\geq$10%) or negative ($<$10%), as previously described.[24]

2.5. Statistical analysis

Disease-specific survival was estimated by using the Kaplan–Meier estimate and compared between subgroups (i.e., MCPyV status; pure vs combined MCC; sun-exposed vs sun-shielded location of the primary tumour) using log-rank test. The clinical features, morphologic features, and immunohistochemical profile of patients between subgroups were compared using the Fisher exact test for categorical variables or independent sample t-test for continuous variables. To investigate the association between the three primary variables of interest and disease-specific survival, we conducted a multivariable Cox model. We evaluated the discriminatory ability performance using the area under the curve, commonly referred to as c-statistics. A two-sided p value <0.05 was considered to be statistically significant. Statistical analyses were performed using SAS version 9.4 (SAS Institute).

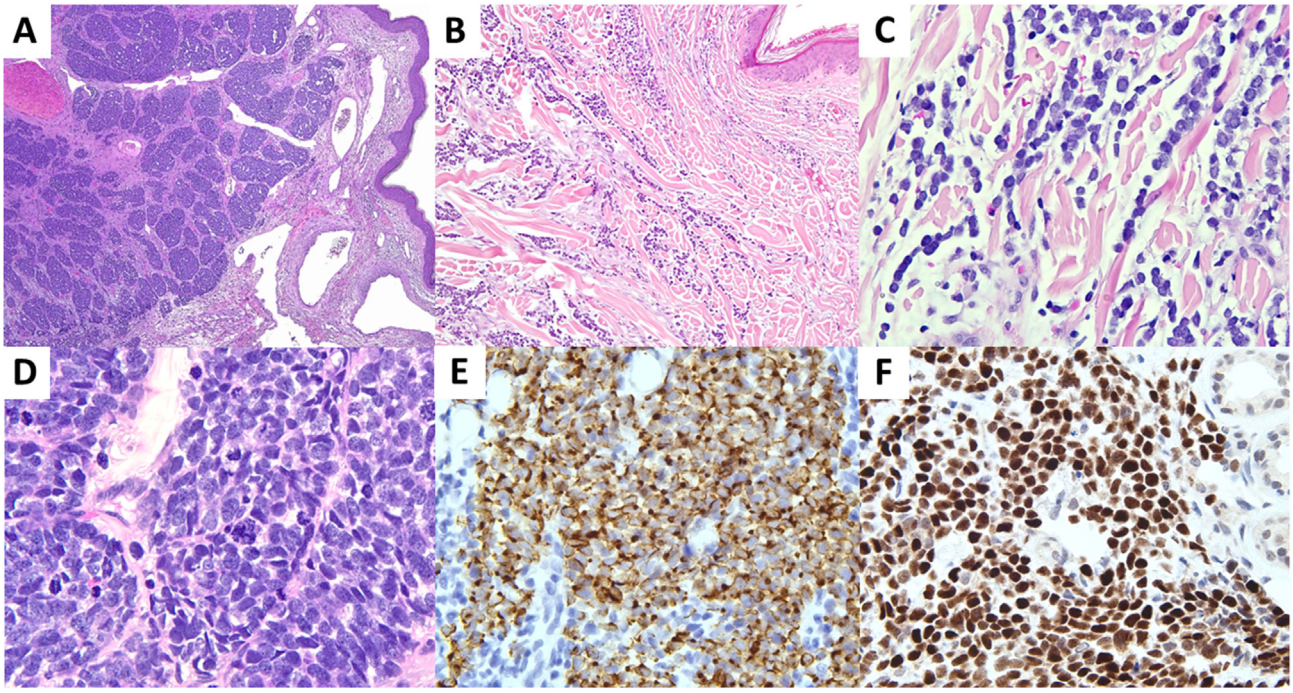

Fig. 1. Morphological and immunohistochemical features of MCC. Various growth patterns including (A) circumscribed and pushing border and (B) irregularly infiltrative pattern (H&E, ×40). (C) A MCPyV-negative tumour with linear or single filing arrangement of tumour cells infiltrating dermal collagen (H&E, ×400). (D) The tumour cells exhibit finely dispersed chromatin and inconspicuous cytoplasm (H&E, ×400). (E) Paranuclear dot-like immunoreactivity with CK20, and (F) homogeneous nuclear expression of MCPyV large T antigen (clone CM2B4, immunohistochemistry, ×400).
H&E, haematoxylin and eosin; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus.

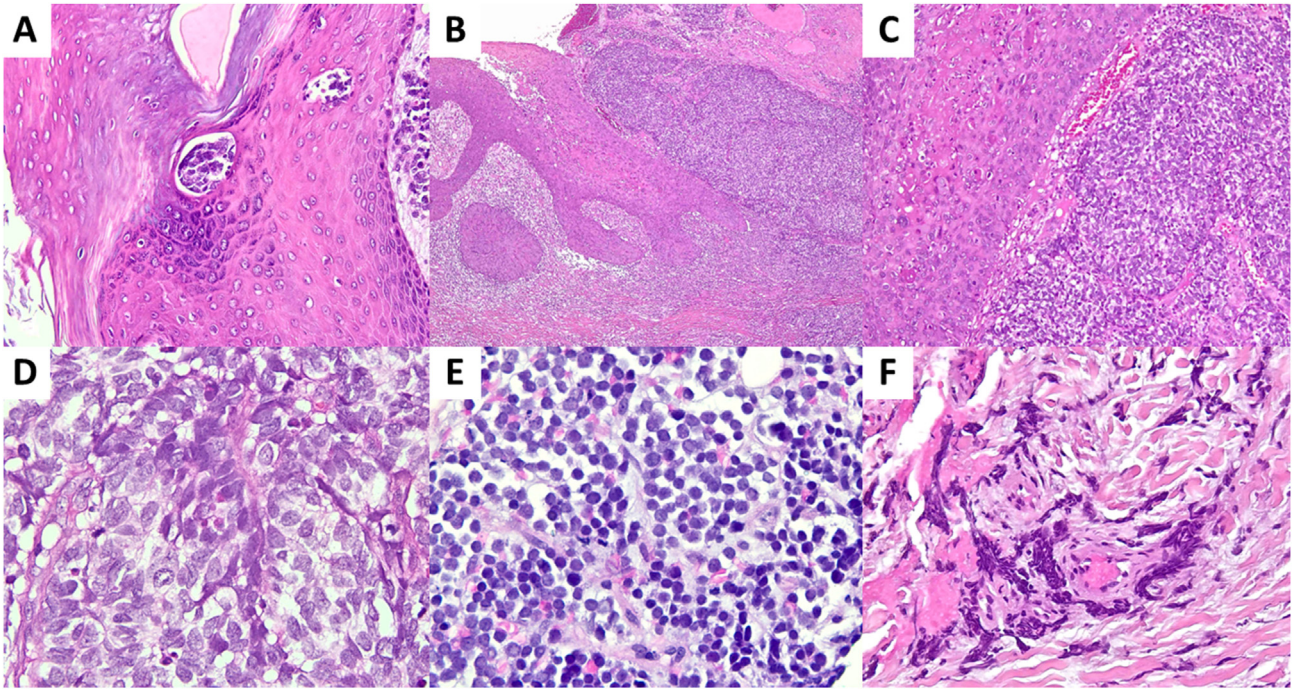

Fig. 2. Various morphological features and combined MCC. (A) Epidermotropism or intraepidermal component of MCC (H&E, ×200). (B) Combined MCCs showing dermal MCC (right) associated with squamous cell carcinoma (left) (H&E, ×40). (C) MCC (right) and squamous cell carcinoma *in situ* (left) in the same tumour without transitional zone (H&E, ×100). (D) A MCPyV-negative case with pleomorphic or elongated nuclei (H&E, ×400). (E) A MCPyV-positive MCC showing small cells with round and regular nuclei (H&E, ×400). (F) Strands or cords of tumour cells with crush artifact (H&E, ×200).
H&E, haematoxylin and eosin; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus.

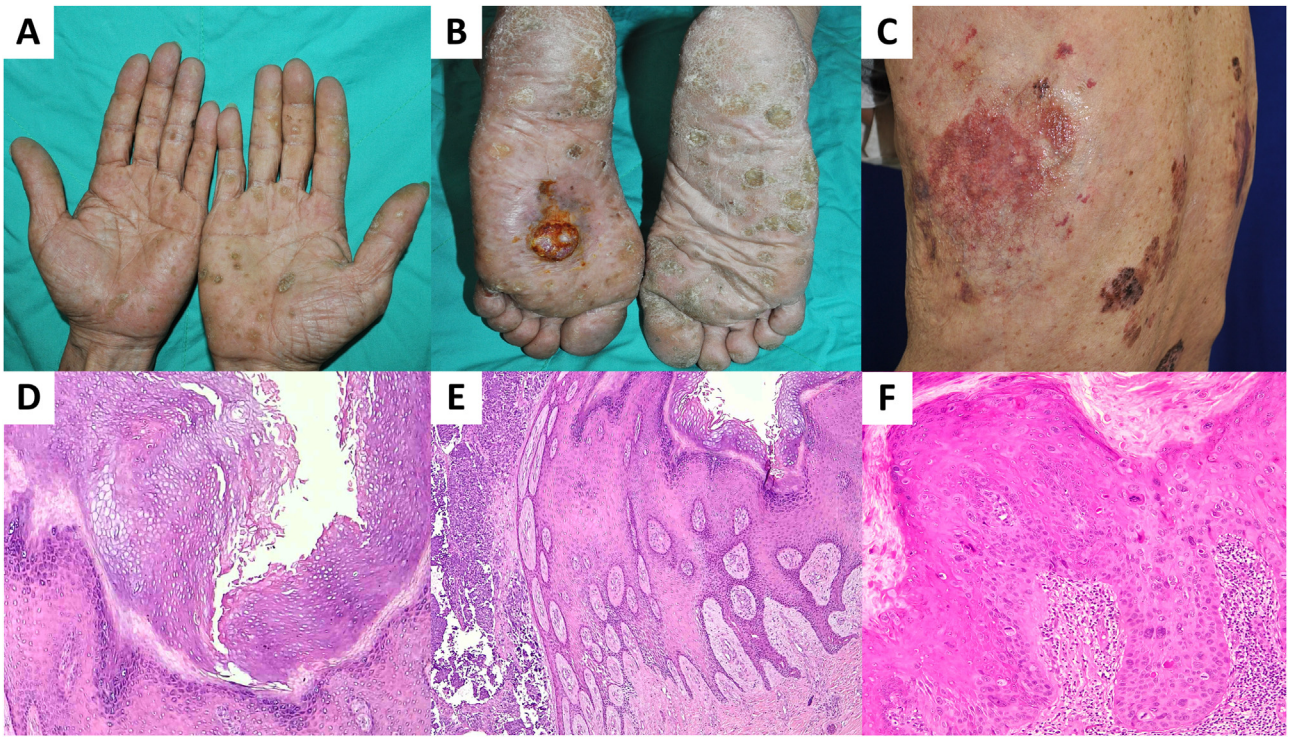

Fig. 3. Cutaneous lesions of two representative cases of MCC associated with chronic arsenicism. (A,B) Case 15 had multiple keratotic papules on his palms and soles, and a combined MCC presented as a reddish tumour with a sharp border. (D,E) Histopathological examination of skin tumour showed epidermal alternations with hyperkeratosis and elongation of rete ridges (H&E; D, ×200; E, ×100). (C) Case 17 had multiple erythematous to brownish patches or macules on the back to chest wall. Combined MCC was developed in one of the plaque-like lesions, with dysplastic change in the squamous component (F, ×200).
H&E, haematoxylin and eosin; MCC, Merkel cell carcinoma.

3. Results

3.1. Patient characteristics and outcome

A total of 38 patients were included, with the clinical information of each patient detailed in Supplementary Table 1 and summarised in Table 1. The median age was 79 years (range 44–97; interquartile range 66–85 years), and the male-to-female ratio was 2.5:1. Five (13%) patients had clinical evidence of chronic arsenicism (Fig. 3), with their clinicopathological features listed in Table 2.

Twenty-two (58%) primary tumours occurred in the sun-exposed sites, and 16 (42%) in the sun-shielded sites. Twenty-seven cases (71%) were pure MCC, whereas 11 (29%) were combined MCCs with concomitant SCC (n=2) or SCIS (n=9). The mean size of primary tumours was 23.3 mm (range 4–80 mm). The disease stage at diagnosis was available for 19 patients, including eight (42%) at stage I, six (32%) at stage II, four (21%) at stage III, and one (5%) at stage IV. The treatment modality was unavailable in one patient, with all other patients undergoing tumour excision. Seven patients received postoperative radiation therapy, and two other patients were treated with both radiotherapy and chemotherapy. Follow-up was available for 35 patients, with a mean of 22 months (range 1–111). Seven (20%) patients had recurrent or metastatic diseases. At the last follow-up, 17 (49%) patients were alive, 15 (43%) died of MCC, three (9%) died of unrelated diseases, and the remaining three (9%) were lost to follow-up. Disease-specific survival of the 35 patients with follow-up information was presented as Kaplan–Meier curves (Fig. 4A). The median overall survival (OS) was 20 months with a 95% confidence interval (CI) of 0–42.3 months (Table 1).

3.2. Histopathology

Under low-power examination, most tumours were dome shaped with an exophytic growth pattern and were located in the dermis, with the larger ones invading the subcutis. Circumscribed tumour borders were found in 13 cases (34%), with infiltrative borders in 11 (29%) and mixed patterns in 14 (27%). Linear growth pattern (strands or cords of tumour cells) was identified in 24 cases (63%). The majority of tumours (29; 76%) did not show crush artifact. Half of the cases (19; 50%) revealed lymphovascular invasion, whereas 12 (32%) had perineural invasion. Lymphocytic response was infrequent (11; 29%).

Eleven cases (29%) were combined tumours with MCC coexisting with SCC (2; 5%) or Bowen disease/SCIS (9; 24%). In one (Case 38), there was a significant deposition of melanin pigment, mimicking melanoma.

Nine (24%) cases showed intraepidermal MCC component. The epidermal changes, in decreasing order of frequency, included hyperkeratosis (45%), ulcer (39%), necrosis (32%), acanthosis/elongation of rete ridges (32%), parakeratosis (29%), and papillomatosis (8%). The uninvolved epidermis displayed dysplastic changes in 12 cases (32%), and basal pigmentation appeared in 10 (26%). Solar elastosis occurred in 23 tumours (61%).

Under high-power examination, small cell and large cell was predominant in 15 (39%) and 23 (61%) cases, respectively. Regular/round nuclei were observed in 14 (37%), with pleomorphic nuclei in 16 (42%), elongated nuclei in two (5%), and mixed components in six cases (16%). In most cases (24; 63%), the cytoplasm was either nearly absent or inconspicuous. The histopathological findings are summarised in Supplementary Table 2.

3.3. Immunohistochemistry

All primary and metastatic tumours met histopathological and immunohistochemical criteria for the diagnosis of MCC. The tumour cells expressed synaptophysin (97%), CK20 (97%), INSM1 (92%), AE1/AE3 (84%), CD56 (82%), and chromogranin A (81%). The MCC component of all 11 combined cases was immunohistochemically positive for at least

Table 1
Summary of clinical and immunohistochemical features of 38 study cases

Variable	Descriptive statistics
Age (years)	
Median [25th, 75th percentiles]	79 [66, 85]
Not available	1
Sex	
Male	27 (71%)
Female	11 (29%)
MCPyV IHC (clone CM2B4)	
Negative	21 (55%)
Positive	17 (45%)
Location	
Sun-exposed site	22 (58%)
Head and neck	11
Extremities	11
Sun-shielded site	16 (42%)
Trunk and thigh	14
Palm and sole	2
MCC subclassification	
Pure MCC	27 (71%)
Combined MCC	11 (29%)
Clinical history of arsenicism	5 (13%)
Greatest dimension of primary tumour, mm	23.3 ± 16.6
Depth of invasion/tumour thickness, mm	11.8 ± 6.2
AJCC stage	
I or II	14 (74%)
III or IV	5 (26%)
Not available	19
Follow-up status	
DOD	15 (43%)
DOUD	3 (9%)
Alive	17 (49%)
Not available	3
Specific death (DOD), month	
KM median survival (95% CI)	20.0 (0–42.3)
Not available	3

Data are presented as frequency (percentage), mean ± standard deviation or median [25th, 75th percentiles].
AJCC, American Joint Committee on Cancer; CI, confidence interval; CM2B4, antibody clone to Merkel cell polyoma virus large T antigen; DOD, died of disease; DOUD, died of unrelated disease; IHC, immunohistochemistry; KM, Kaplan–Meier; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus.

one of the four NE markers (synaptophysin, chromogranin A, INSM1, and CD56). Only two MCCs, including one combined tumour, exhibited aberrant expression of TTF-1 (clone SP141), as previously reported by Creytens.[25] Neither patient had clinical or imaging evidence of lung cancer. Seventeen (45%) tumours were positive for MCPyV with a nuclear staining pattern (Fig. 1C). MCPyV was negative in all the combined MCCs and all the five arsenic-related tumours. Supplementary Table 3 summarises the immunohistochemical findings.

All cases were immunostained for Rb except Case 4. Of the 37 cases, 15 (41%) showed retained expression in virtually all tumour cells, whereas 22 (59%) showed complete loss of Rb protein, with an example shown in Fig. 5. The association of Rb protein expression with various factors is summarised in Supplementary Table 4. MCPyV-unrelated MCC cases more frequently lost Rb expression (18/21 or 86% vs 4/16 or 25%) than MCPyV-related cases ($p<0.001$; Fisher exact test). Combined MCC cases more frequently lost Rb expression (10/11 or 91% vs 14/26 or 54%) than pure MCC cases ($p=0.014$; Fisher exact test). Among all the 11 cases of combined MCC stained for Rb, in one case (Case 12) there was no more SCC/SCIS component in the Rb-stained section for evaluation. Of the remaining 10 cases with combined MCC, in one case (Case 17) Rb expression was retained in the MCC component but lost in the SCIS component, whereas in the remaining nine (90%) cases, Rb expression was lost in both the MCC and SCC/SCIS components ($p=1.000$). There was no statistically significant difference in Rb loss in terms of arsenic exposure [4/5 (80%) in arsenic-related vs 18/32 (56%) in arsenic-unrelated cases; $p=0.629$] or sun exposure [8/15 (53%) in sun-exposed vs 7/22 (32%) in sun-protected cases; $p=0.307$]. Finally, Rb expression was not related to disease-specific survival ($p=0.180$).

3.4. *Sun-exposed versus sun-shielded sites*

Histologically, the sun-exposed group had more frequent expansile growth pattern and solar elastosis ($p=0.049$ and $p<0.001$, respectively) than the sun-shielded group, without statistical difference in the immunohistochemical findings (Supplementary Tables 4 and 5). The former group of patients more frequently had low-stage diseases (92% vs 33%; $p=0.017$; Supplementary Table 6) and a better disease-

Table 2
Clinicopathological features of the five chronic arsenicism-related MCCs

Case no./sex/age	Location of MCC	Pure vs combined MCC	Stage	Other associated cutaneous lesions indicating chronic arsenicism	Treatment	FU (months)
13/M/79	Right lower back	Pure	T2N1M0	Multiple erythematous to pigmented, crusted plaques (Bowen disease) on trunk and limbs	Excision and R/T	DOD (8)
15/M/59	Left sole	Combined with SCIS	T1N1bM1a	1. Multiple erythematous to pigmented, crusted plaques (Bowen disease) on trunk and limbs 2. Multiple punctate keratotic papules on palms and soles	Excision and R/T	DOD (3)
17/M/79	Back	Combined with SCIS	T2N1bMx	1. Multiple erythematous and brownish crusted plaques (Bowen disease) over trunk and upper limbs 2. Freckled raindrop pattern with hyperpigmented and hypopigmented macules on trunk 3. Multiple punctate keratotic papules on palms and soles	Excision and R/T	DOD (8)
22/F/87	Scalp	Pure	T1N0M0	Multiple erythematous to pigmented crusted plaques (Bowen disease) on face and scalp	Excision	NED (60)
23/F/88	Right chest	Combined with SCIS	T1N0M0	Multiple erythematous to pigmented crusted plaques (Bowen disease) on limbs	Excision and C/T	DOD (10)

C/T, chemotherapy; DOD, died of disease; FU, follow-up; MCC, Merkel cell carcinoma; NA, not available; NED, no evidence of disease; R/T, radiotherapy; SCIS, squamous cell carcinoma *in situ*.

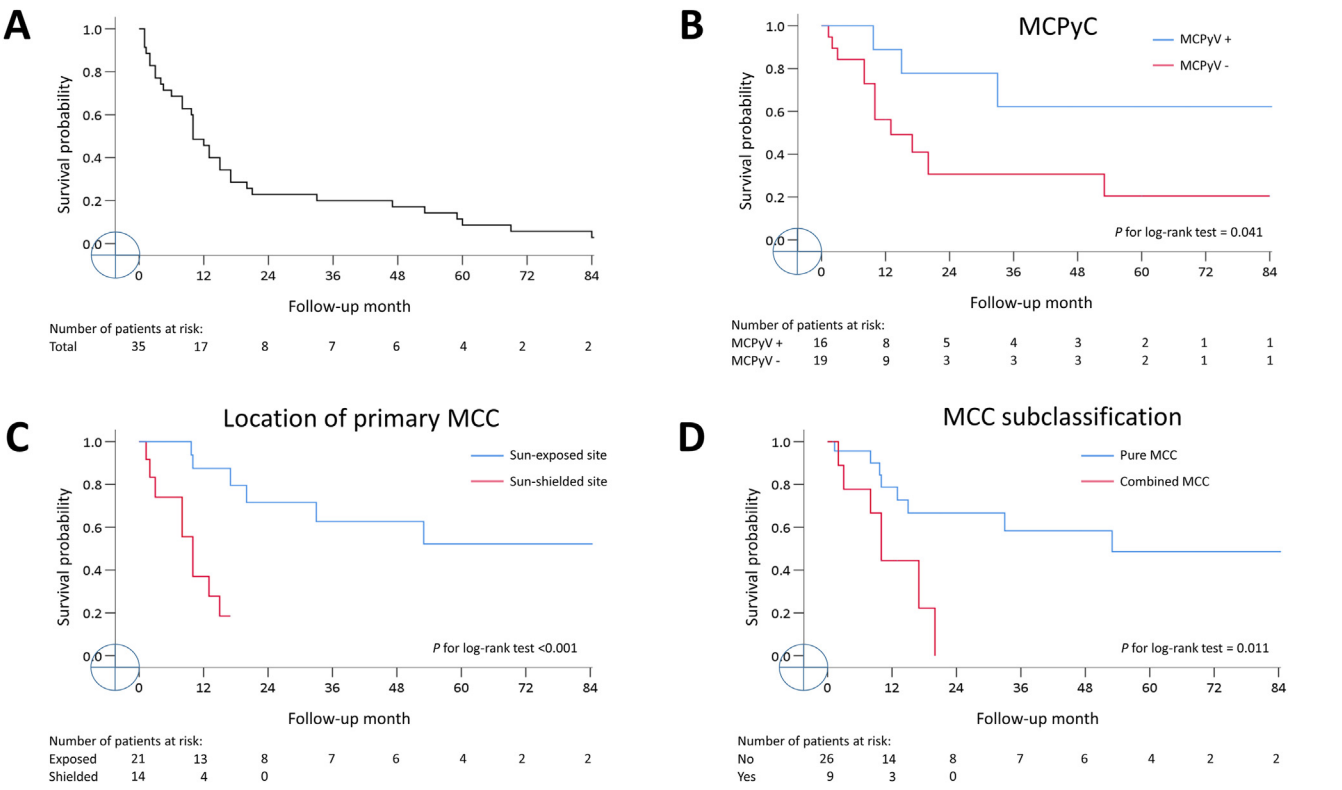

Fig. 4. (A) The Kaplan–Meier survival curves of disease-specific survival of the 35 MCC patients with available follow-up data and (B) further stratified by the presence or absence of MCPyV, (C) location, and (D) combined or pure MCC.
MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus.

specific survival (median OS not reached vs 10 months, $p<0.001$; Fig. 4C) than the latter group.

3.5. MCPyV-positive versus MCPyV-negative cases

We found that MCPyV-positive ($n=17$) and MCPyV-negative ($n=21$) cases were morphologically distinct (Supplementary Table 5). All MCPyV-positive cases were pure MCCs, whereas approximately half (52%; 11/21) of the MCPyV-negative cases were combined MCCs ($p<0.001$) (Table 2). The epidermal alterations (ulcer, necrosis, hyperkeratosis, parakeratosis, acanthosis, and elongation of rete ridges) were more common in MCPyV-negative cases ($p=0.002$, $p=0.004$, $p=0.006$, $p=0.009$, and $p<0.001$, respectively; Supplementary Table 5). Of note, virtually only MCPyV-negative MCCs harboured an intraepidermal MCC component ($p=0.048$) and displayed squamous cell dysplasia ($p<0.001$). Furthermore, linear or single-file arrangement ($p=0.018$), lymphovascular invasion ($p=0.049$), and pleomorphic nuclei ($p=0.008$) were more frequently observed in MCPyV-negative cases, whereas regular or round nuclei were more common in MCPyV-positive MCCs ($p=0.008$; Supplementary Table 5) Immunohistochemically, there was no significant difference in the expression rates of various markers between these two groups (Supplementary Table 7).

MCPyV-positive cases had a better disease-specific survival than MCPyV-negative MCCs (median OS not reached vs 13 months, $p=0.041$; Fig. 4B). There was no significant difference between MCPyV-positive and MCPyV-negative cases in terms of age, sex, tumour location, tumour size, and AJCC stage (Table 2).

3.6. Combined versus pure MCC

Histologically, the MCC component in combined MCC cases showed certain histological features distinct from those in the pure MCC cases (Supplementary Table 4). All 11 combined MCCs were negative for MCPyV, as compared to only 37% (10/27) MCPyV-negative pure MCC cases ($p<0.001$) (Table 2). Combined MCC was significantly associated with poor disease-specific survival as compared to pure MCC (median OS 10 months vs not reached, $p=0.011$; Fig. 4D).

3.7. Survival analysis

We examined the prognostic significance of three factors: MCPyV status, pure vs combined subgroup, and sun-exposed or sun-shielded location (Supplementary Table 4). The multivariable Cox model revealed that the presence of MCPyV was not independently associated with disease-specific survival [hazard ratio (HR) 0.59, 95% CI 0.14–2.5, $p=0.480$] with adjustment of the other two variables (data not shown). Both combined MCC (HR 4.50, 95% CI 1.22–16.60, $p=0.024$) and sun-shielded location (HR 10.97, 95% CI 2.64–45.63, $p<0.001$) were independently associated with poor survival (data not shown). The performance of the joint effect by the three primary variables of interest in discriminating disease-specific survival was satisfied with the c-statistics of 79% (95% CI 61.9–90.9%) (Fig. 6).

4. Discussion

To our knowledge, our current study is the most comprehensive clinicopathological investigation of MCC in Taiwan. Nearly half (45%) of the tumours were positive for MCPyV. Interestingly, all combined cases ($n=11$; 29%) were negative. As compared to MCPyV-positive cases, MCPyV-negative MCCs displayed distinctive pathological features and a shorter disease-specific survival. In addition, MCPyV-negative and combined MCCs were associated with a higher disease-specific mortality and poorer prognosis. MCCs in sun-shielded sites, MCPyV negativity, and combined tumours correlated with a higher disease-specific mortality and a poorer prognosis. Additionally, we found that a subset of our patients were chronic arsenicism-related.

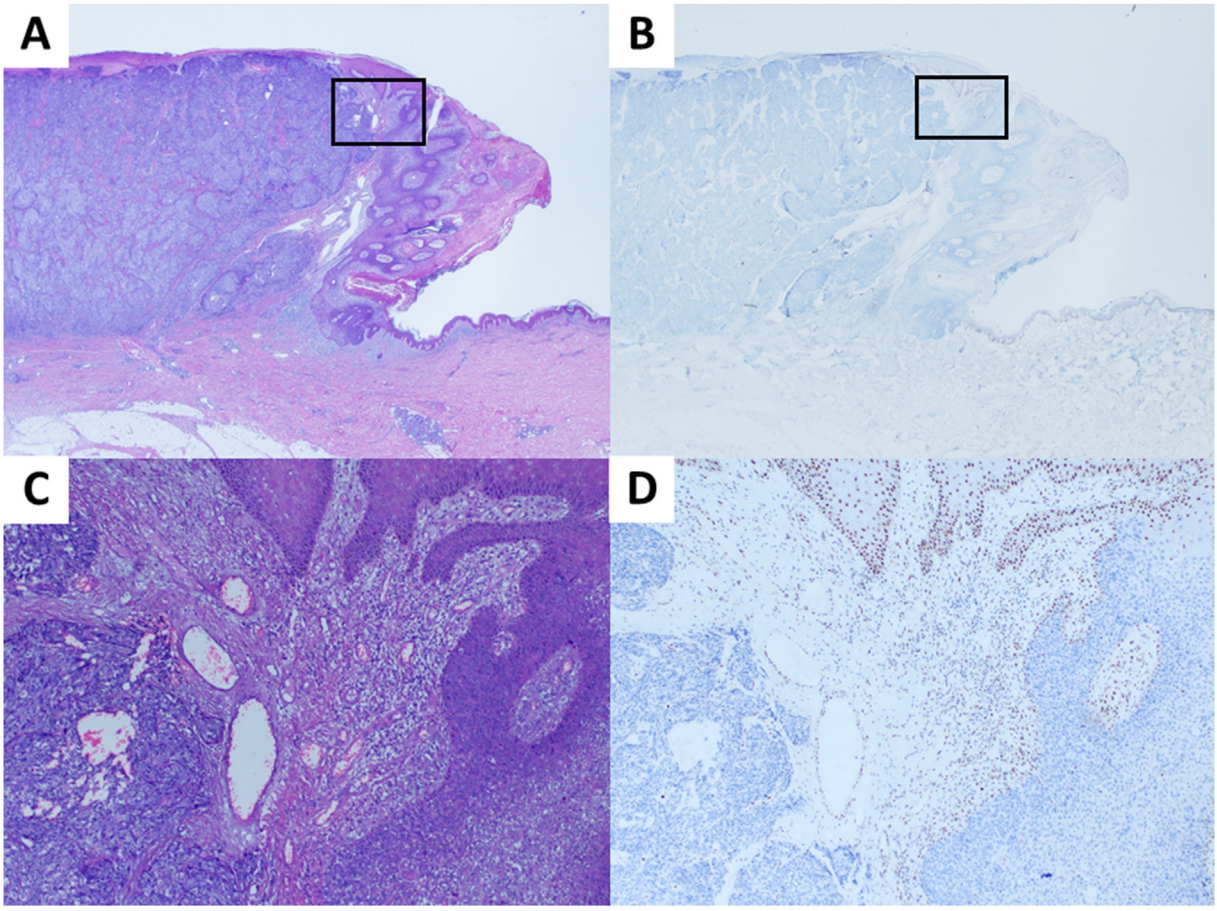

Fig. 5. Case 22, a case of mixed MCC. The exophytic tumour shows the MCC component in the left-hand side, the SCIS part in the right-hand side, and the hyperplastic epidermis between these two components (H&E; A, 12.5× and C, 100×). Immunohistochemical stain with Rb shows complete loss of Rb expression in both tumor components, while the hyperplastic epidermal cells in the upper part of panel D retain normal Rb expression (A,C, H&E; B,D, Rb stain; A,B, 12.5×; C,D, 100×; representing the enlarged rectangular area of A and B, respectively). H&E, haematoxylin and eosin; MCC, Merkel cell carcinoma; SCIS, squamous carcinoma *in situ*; Rb, retinoblastoma.

MCC lesions may be circumscribed or highly infiltrative.[26] In our study, 29% cases showed a pure infiltrative pattern with neoplastic cells infiltrating between dermal collagen bundles with linear strands or 'single-file' arrangement. Involvement of the epidermis (epidermotropism or intraepidermal component) is uncommon but has been well documented.[11,27] We observed this phenomenon in 24% of our cases. Most MCCs exhibited a NE morphology, with a high nuclear-to-cytoplasmic ratio, round-to-ovoid nuclei with so-called salt and pepper chromatin, nuclear moulding, and a high mitotic rate. In the current study, we investigated the significance of MCPyV status, pure or combined subtype, and primary tumour location on the pathological features of MCC. We found a considerable morphological difference between MCPyV-positive and MCPyV-negative MCCs. MCPyV-positive cases were characterised by regular and round nuclei, with absence of an intraepidermal component. In contrast, the MCPyV-negative samples had more pleomorphic nuclei, more frequently with an intraepidermal component. We also found that linear strands or 'single-file' pattern, ulcer or necrosis, and lymphovascular invasion were more frequent in MCPyV-negative tumours. In addition, MCPyV-negative MCCs more frequently had epidermal alternations (hyperkeratosis, parakeratosis, and elongation of rete ridges). Such epidermal alternations were also statistically significant between the pure and combined MCC groups. In contrast, there were no such differences between the sun-exposed and sun-shielded subgroups, except that expansile tumour border and solar elastosis were more common in the sun-exposed cohort. There was no statistically morphological difference in terms of the expression of diagnostic immunohistochemical markers.

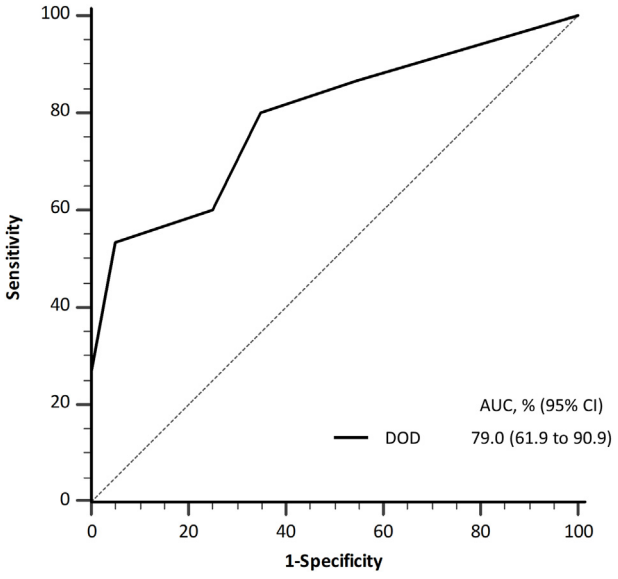

Fig. 6. The joint effect of the three primary variables of interest in discriminating disease-specific survival among the 35 MCC patients with available follow-up data. AUC, area under the curve; CI, confidence interval.

MCC is a rare and aggressive cutaneous NE carcinoma with two major aetiological factors: MCPyV and long-term UV light exposure.[3,9–11] The other reported risk factors include white skin type, old age, male sex, and immunosuppression.[3,9,11,28] Prior research has shown that MCCs can be categorised into two distinct molecular classes. MCPyV-negative MCCs have high mutational burdens characterised by UV-signature events, supporting UV-induced damage as an aetiology. In contrast, MCPyV-positive MCCs harbour relatively few mutations and do not display a definitive UV signature, supporting an oncogenic role for MCPyV T antigens as primary drivers for these tumours.[9,11,28–30] More recent investigations reveal that MCPyV-positive and MCPyV-negative MCCs are characterised by unique morphological features, suggesting a major variation in tumour biology and behaviour.[20,24] In our previous report and those from other groups, MCPyV-negative tumours have been found to carry a worse prognosis than those positive for MCPyV.[16,31,32]

Arsenic poisoning is a systemic disease and can develop from environmental, occupational, and medicinal exposure. Long-term exposure to inorganic arsenic can lead to the development of certain cutaneous lesions, blackfoot disease, and various cutaneous, visceral and haematological malignancies.[22,33–35] In Taiwan, there were two endemic regions where residents had a long-term exposure to inorganic arsenic from drinking water. Those living in the southern and northeastern endemic areas began drinking from high-arsenic artesian well water in the early 1910s and late 1940s, respectively, and that lead to the development of blackfoot disease, an endemic peripheral vascular disease or chronic arsenic poisoning leading to necrosis of the extremities.[34] Starting from the early 1990s, sporadic reports of MCC cases from patients living in the endemic areas of blackfoot disease emerged.[14,15,36,37] In the late 1990s, there were also sporadic cases of MCC developing in Japanese patients with chronic arsenicism.[38,39] In our current study, five (13%) patients had chronic arsenicism. There were various cutaneous non-neoplastic lesions (arsenical dermatosis) associated with chronic arsenic toxicity, such as the raindrop pattern with hyperpigmented and hypopigmented macules, hyperkeratosis, acanthosis or papillomatosis, and squamous dysplastic change (Fig. 3 and Supplementary Table 2).[22,23] Interestingly, these five MCCs were all negative for MCPyV. Three of these cases were combined MCC, all with concomitant Bowen disease, consistent with chronic arsenicism. A recent population-based study investigating the impact of a tap water supply system installation beginning in 1960s in blackfoot disease-endemic regions in Taiwan revealed a reduced burden of several arsenic-related cancers including Bowen disease between 1995 and 2019.[40] MCC was not included in that study, probably because the researchers in that study were not aware of the association of chronic arsenicism with MCC. We expect that the incidence of arsenic-related MCC will be decreasing in Taiwan due to the installation of tap water in the endemic regions.

In a review of environmental cancers in Taiwan, Yu *et al.* reported that arsenical skin cancers usually present as multiple lesions at different disease stages.[33] The skin cancers frequently occur in sun-shielded areas as UVB exerts an inhibitory effect on the proliferation of arsenical cancers.[33] In our study, four of our arsenic-related MCC cases were located in sun-shielded areas (Table 2). Ho *et al.* reviewed 10 patients from arsenic endemic areas and suggested that arsenic-related combined MCCs tend to occur in sun-shielded areas, making this entity clinically distinct from UV-related combined SCC and MCC.[36] Hsu *et al.* examined arsenic-related skin malignancies in the endemic area of Taiwan and discovered a significantly high rate of *p53* mutation, with mutation types distinct from those in UV-induced skin malignancies.[41] In our current study, we noted that chronic arsenicism-related MCCs were frequently MCPyV negative. These data suggest that arsenic intoxication-related MCCs are distinct from UV-related MCC in pathogenesis. In our subsequent research, we will be investigating the genetic alterations to elucidate the pathogenesis of MCC in Taiwan.

Combined MCCs account for 5–20% of all MCCs, most commonly in association with Bowen disease or invasive SCC, but other carcinomatous or sarcomatous components have been reported.[11,16,18,19,21,42–46] There are higher frequencies of metastasis and death in patients with combined MCC than in those with pure MCC.[18,42,43,45,46] In accordance with the previous studies, we found that the group of 11 combined MCC cases in our study was associated with a higher rate of MCC-specific death (78%) than the pure MCC group.

The *RB1* gene encodes the Rb protein, a tumour suppressor, by inhibiting cell cycle. In our current study, Rb protein loss is more frequent in MCPyV-negative cases, which is in line with the previous studies.[47,48] Additionally, in combined cases, both the SCIS/SCC and MCC components lost Rb protein expression, similar to the recent studies suggesting that *RB1*-deficient dysplastic squamous cells might be the source of combined MCC.[11,49,50] In our study, there is no significant association of Rb loss and chronic arsenicism. We speculate that due to the retrospective nature of our study, some chronic arsenicism-related cases might not have been identified and were classified as chronic arsenicism-unrelated. With the decreasing incidence of blackfoot disease in Taiwan due to improved public health, further studies on the association with chronic arsenicism might be very challenging or not possible.

The major limitations of our study are the relatively limited case number, the retrospective nature, and the multicentre design. Due to the rarity of this tumour, there is no established management protocol in Taiwan. The possible coexistence of cutaneous changes associated with arsenicism might be overlooked and/or unrecorded. Furthermore, the treatment modality was heterogeneous, which certainly had an impact on survival. Nonetheless, our study is the first comprehensive clinicopathological analysis of MCC in Taiwan.

5. Conclusions

In this first comprehensive and retrospective study of MCC in Taiwan, we found that MCPyV-negative cases exhibited distinctive pathological features and a poorer outcome than MCPyV-related cases. We also identified an association of chronic arsenicism with MCC, which might be considered as the third pathogenetic factor after MCPyV and UV light. A national registry of MCC should be established, and further studies including epidemiological and genetic investigations are warranted to elucidate the pathogenesis of MCC in Taiwan, particularly the significance of chronic arsenicism.

Conflicts of interest and sources of funding

The authors state that there are no conflicts of interest to disclose.

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Supplementary data

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