

## Isolation of a Coronavirus from Urinary Tract Tumours of Endemic Balkan Nephropathy Patients

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Dear Sir,

Endemic Balkan nephropathy (EBN) is a mysterious disease prevalent in recognised areas of three countries: former Yugoslavia, Rumania and Bulgaria. The endemic localities are found along the valleys of the rivers Danube, Sava and their tributaries. The patients are mainly rural people over 40 years old. The cardinal symptoms are progressive loss of kidney function without raised blood pressure leading to end-stage kidney failure. In up to 50% of EBN patients, tumours of the urinary tract appear [1]. It has been estimated that there have been more than 15,000 cases of EBN in the 3 countries. At present there are over 700 EBN patients on dialysis in Serbia alone. Toxins, including heavy metals and mycotoxins have been suggested as a cause. Evidence for the involvement of coronaviruses based on histological and ultrastructural studies has been published [2, 3]. However, it is only recently that a coronavirus was isolated from kidney biopsies [4]. The isolation of the virus (EBNV) and serological investigations suggest its involvement in the aetiology of EBN. This is further supported by the isolation of an identical coronavirus from urinary tract tumours and a metastatic lymph node.

**Table 1.** Neutralisation antibody titres to EBNV in EBN patients and controls

Patients	n	Serum dilution							total +	%
		1/16	1/32	1/64	1/126	1/256	1/512			
EBN on dialysis	65	12	6	12	18	7	2	57	87.7	
On dialysis, no EBN	12	2	0	0	0	0	0	2	16.6	
Tumour and EBN	13	0	0	1	3	4	5	13	100	
Tumour nonendemic	13	0	1	0	0	0	0	1	7.7	

Tumour, kidney tissue and a metastatic lymph node were obtained at operations on 5 EBN patients from the endemic region of Doboj, Bosnia. A coronavirus was isolated from the kidneys as described previously [4]. The tumour tissue and the lymph node were stored at  $-20^{\circ}\text{C}$ . After thawing and the establishment of viable cell cultures they were co-cultivated with Vero cells. An agent was isolated from all five tumour specimens and significantly also from a patient's metastatic lymph node. Neutralisation and immunofluorescence tests indicated that the agent is a

coronavirus identical to EBNV isolated from the kidneys of the same patients [4]. The virus could also be isolated in Vero cells from the supernatant of the tumour cell cultures as well as from the lymph node suspension cultures. In order to exclude cross-contamination, in a separate experiment EBNV hyperimmune serum was added to a primary tumour cell culture and incubated for several days. On removal of the medium, washing and re-incubation, the virus was repeatedly isolated in Vero cells. Some of the serological investigations are summarised in table 1 up-

dating the one previously published [4]. It is seen that in neutralisation tests 87.7% of the EBN patients on dialysis were positive, while all 13 EBN patients with tumours of the urinary tract were positive. In the single positive case of a tumour without EBN, the patient has lived in an endemic locality.

We have also confirmed that the tumours in situ and in culture were positive by immu-

nofluorescence of the hyperimmune serum raised with EBNV [4].

The isolation of a coronavirus from a tumour is surprising. Coronaviruses have not been shown to be associated with tumours. The results presented here and those from a previous report reinforce the notion of a causal relationship between EBN and EBNV.

## References

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- 3 Holmes KV, Lai MC: Coronaviridae: The viruses and their replication; in Fields BN, Knipe DM, Howley PM, et al (eds): *Fields Virology*, ed 3. New York, Raven Press, 1996, p 1075.
- 4 Uzelac-Keserović B, Spasić P, Bojanić N, et al: Isolation of a coronavirus from kidney biopsies of endemic balkan nephropathy patients. *Nephron* 1999;81:141–145.