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# Hepatitis C-associated B-cell non-Hodgkin lymphomas: The emerging role of miRNA-26b

*To the Editor:*

We read with interest the review by Peveling-Oberhag *et al.* [1] that provides a very exhaustive picture of the knowledge about HCV-related B-cell non-Hodgkin lymphomas (NHL), from its connection with the mixed cryoglobulinemia (MC) condition and epidemiology to pathomechanisms, passing through therapeutic options.

In spite of the multiple pathogenetic hypotheses proposed for HCV-related NHL [2], since the link between malignancy and infection was shown [3–5], the authors clearly explained the research advances, summarizing them in three different theories: the sustained B cell stimulation, the direct oncogenic potential of viral proteins, and the genetic virus-induced damage by a so called "*hit and run*" action. Interestingly, the authors include microRNA dysregulation in the dissertation about the first theory, suggesting a key role of miR-26b downregulation in undermining tumor suppression [6]. We could recently confirm these data on NHLs and also, consistently increase the potential importance of miR-26b levels in determining HCV-related lymphoproliferation [7]. In fact, our results showed miR-26b downregulation not only in NHL, but also in MC patients, suggesting its involvement also in this condition, considered to be a prelymphomatous disorder. More interestingly, in patients who, after antiviral therapy, experienced viral eradication, the MC syndrome disappeared and miR-26b expression was restored to normal levels. From a translational point of view, we could show the usefulness of miR-26b detection in PBMCs, obtainable through non-invasive blood sampling; this was especially interesting in consideration of the difficulties in detecting miR-26b modifications in serum samples of the same MC or NHL patients (personal, unpublished data), making the PBMC test the easiest way to evaluate this potential marker through a simple blood drawn. The following, so far unexplored, step will therefore be the identification of miR-26b target genes; Peveling and colleagues already suggested the *NEK6* gene coding for a kinase involved in the initiation of mitosis [6]. In addition, we proposed the lymphoid enhancer factor 1 (*LEF-1*) gene [7]. LEF-1 is a nuclear transcription factor transiently expressed in the pro-B and not detectable in mature B cells, but recently indicated as a specific target of miR-26b [8]; previous studies showed elevated levels of LEF-1 in human cancers, in chronic lymphatic leukemia [9] and in a wide cohort of diffuse large B cells lymphomas [10], making this a potential target of special interest in the pathogenetic research field.

In conclusion, our results on HCV positive MC strengthen previous data on the importance of miR-26b modulation in HCV-related NHL, opening new perspectives for further studies on target genes and also aimed to a better definition of this microRNA as disease biomarker.

## Conflict of interest

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# Long-term protection of neonatal hepatitis B vaccination in a 30-year cohort in Hong Kong

*To the Editor:*

Universal neonatal hepatitis B (HB) vaccination programme is effective in the control of hepatitis B virus (HBV) infection in countries with different endemicity [1,2]. However, the long-term protective effect of neonatal hepatitis B vaccination into adulthood, especially in endemic areas and to subjects with high risk of infection, remains uncertain [3]. Universal neonatal HB vaccination programme has been introduced in Hong Kong, a region of moderate to high endemicity of hepatitis B, since 1988. To study the long-term effect of neonatal HB vaccination in those with high risk of HBV infection in Hong Kong, we evaluated the serial changes of HBV serological markers over a 30-year period in a cohort born to hepatitis B carrier mothers and who received neonatal hepatitis B vaccination.

In 1983, 1112 neonates, born to hepatitis B carrier mothers in a public hospital in Hong Kong, were recruited for a study to evaluate the response of passive–active hepatitis B vaccination with hepatitis B immunoglobulin and three-dose regimen of hepatitis B vaccines of three different schedules: conventional (0, 1, and 6 months), delayed (2, 3, and 8 months), and accelerated (0, 1, and 2 months) [4]. HBV serological markers, including HBsAg, antibody to surface antigen (anti-HBs) and antibody to core-antigen (anti-HBc), were determined upon completion of vaccination and at subsequent intervals up to 30 years of follow-up. Anti-HBs positivity was defined as anti-HBs level higher or equal than 10 IU/L. Anti-HBc seroconversion was defined as anti-HBc positivity for two consecutive readings measured at or after the 2nd year. Upon completion of the three-dose vaccination, 1006 (92.6%) out of 1086 subjects developed anti-HBs positivity. Thirty-nine subjects failed the vaccination and developed chronic HBV infection, giving the HBsAg positivity rate of 3.5%. Thirty-five (89.7%) of them were born to mothers who were also hepatitis B e-antigen (HBeAg) positive. All of these 39 subjects were tested HBsAg positive before the age of two, with no new infection found at subsequent follow-up time points of years 3, 5, 7, 10, 13, 16, 21, 25, and 30 post-vaccination (Table 1). At the 30th year of follow-up, the anti-HBs positivity rate fell to 37.4%. There were no differences in the development of HBsAg and anti-HBs positivity between subjects receiving three different vaccination schedules ($p$ >0.05). Ninety-seven subjects developed anti-HBc seroconversion over the 30-year period, giving a rate of 9% (39 subjects tested for HBsAg positive were excluded), with no statistically significant difference in anti-HBs positive and negative subjects (9.3% *vs.* 5%, $p$ >0.05).

We described the first study showing long-term protective effect of neonatal HB vaccination into a cohort up to 30 years of follow-up. While most studies reported in the literature measured immune-protection by detectable levels of antibodies to HBV [5,6], this study provided direct evidence of effective protection in subjects with high risk of infection, both via perinatal route or close interpersonal contact with their infected mothers and possibly other infected household members at childhood. Among 97 subjects who developed anti-HBc seroconversion, a majority (43%) of these seroconversions occurred at or before year two. On the other hand, with such a long follow-up period up to 30 years, this study also demonstrated the protective effect of vaccine into adulthood, when the subjects might be exposed to horizontal transmission of HBV, notably from sexual contact. Despite the dropping rate of anti-HBs and new occurrence of anti-HBc seroconversion over the years, there was no new development of HBsAg positivity after the first two years in the entire follow-up period.

Our findings were in line with other studies which suggested long-term protection of HB vaccine albeit with shorter follow-up durations. Persistence of serum protective antibody level was shown in older children or adolescents who received vaccination at infancy [6–8]. Moreover, anamnestic response was demonstrated in those who had lost protective antibody level, years after vaccination [9]. A recent study further suggested that, apart from a protective serum antibody level, cellular immune response might also play a role for protection against HBV after vaccination [10].

There were two limitations in this study. First, subjects who were not followed-up might have affected the overall representativeness of the data. At the 30th year of follow-up, 246 (22.1%) subjects returned for serological tests. 372 (33.5%) subjects defaulted follow-up, and 492 (44.2%) subjects were lost to fol-