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Levamisole improves immune response to hepatitis B vaccine in dialysis patients: Meta-analysis of randomized trials

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LEVAMISOLE IMPROVES IMMUNE RESPONSE TO HEPATITIS B VACCINE IN DIALYSIS PATIENTS: META-ANALYSIS OF RANDOMIZED TRIALS

Short Title: Levamisole and hepatitis B vaccine in dialysis

Fabrizio Fabrizi,¹⁻² M.D., Vivek Dixit,² Ph.D., Piergiorgio Messa,¹ M.D.,
and Paul Martin,² M.D.

Division of Nephrology and Dialysis,¹ Maggiore Hospital, IRCCS Foundation, Milano, Italy; and
Division of Hepatology,² School of Medicine, University of Miami, Miami, Florida, U.S.

Address for correspondence: Dr. Fabrizio Fabrizi, Divisione Nefrologica, Ospedale Maggiore, Pad. Croff, Via
Commenda 15, 20122, Milano, Italia

Phone: (39) 2 55034553; Fax: (39) 2 55034550; E-mail: fabrizi@policlinico.mi.it

ABSTRACT

Purpose: Patients undergoing maintenance dialysis often fail to mount protective antibodies to hepatitis B virus surface antigen (HBsAg) following vaccination against hepatitis B virus (HBV). Some authors have suggested that levamisole improves immune response to HBV vaccine in dialysis population. However, consistent information on this issue does not exist. We evaluated efficacy and safety of levamisole as adjuvant to hepatitis B virus (HBV) vaccine in dialysis patients by performing a systematic review of the literature with a meta-analysis of clinical trials.

Methods: We used the random effects model of DerSimonian and Laird, with heterogeneity and sensitivity analyses. Only trials comparing the seroresponse rate in study subjects (levamisole plus HBV vaccine) *versus* controls (HBV vaccine alone) were included. The end-point of interest was the rate of patients showing seroprotective anti-hepatitis B titers at completion of HBV vaccine schedule in study versus control groups.

Results: We identified four studies involving 328 unique patients on regular dialysis. Only prospective, randomized clinical trials (RCTs) were included. Pooling of study results showed a significant increase in response rates among study (levamisole plus HBV vaccine) *vs.* control (HBV vaccine alone) patients; the pooled Odds Ratio was 2.432 (95% Confidence Intervals, 1.34; 4.403), $P=0.002$. No study heterogeneity was found. These results did not change in various subgroups of interest.

Conclusions: Our meta-analysis showed that levamisole significantly improve immune response to hepatitis B vaccine in dialysis population. The limited number of patients precluded more conclusions.

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KEY-WORDS

Hepatitis B virus Vaccine; Levamisole; Dialysis; Meta-analysis

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INTRODUCTION

Hepatitis B Virus (HBV) infection is a serious global health problem with more than 150 million chronic carriers. The frequency of hepatitis B infection has been higher in dialysis patients than in the general population because of their potential constant exposure to blood, frequent transfusions, and sharing of dialysis equipment [1]. The Centers for Disease Control and Prevention recommends that all dialysis patients should be vaccinated against HBV, and vaccination is strongly recommended for patients with chronic kidney disease (CKD), dialysis-dependent or not, who are candidates for kidney transplantation [2]. Outbreaks of HBV infection among the haemodialysis patients continue to be recognized in the industrialized countries [3-5]

It has been repeatedly noted that immune responses to hepatitis B vaccination are impaired among chronic kidney disease populations, proportionally to the degree of kidney failure. Patients with chronic kidney disease experience lower seroconversion rates (40-70%), lower peak antibody titers, and shorter duration of seroprotection [6]. The impaired efficacy of HBV vaccination in dialysis patients has been attributed to numerous factors notably immune compromise because of uraemia, age [7], gender [8], body weight [9], nutritional status [10], serological positivity for HCV [11] or HIV [12] infection, blood transfusion history [13], and possession of the major histocompatibility complex haplotype HLA-B8, SC01, DR3 [14]. Also, the failure to complete a full course of HBV vaccination impairs protective efficacy [15].

Various approaches have been adopted to improve the response rate to hepatitis B vaccine in end-stage renal disease including the increased vaccine dose [16], additional vaccine inoculations, and the use of intradermal rather than intramuscular vaccine route [17]. HB vaccination in chronic uraemic patients not yet requiring the dialysis results in seroprotection rates higher than in dialysis patients [18]. Additional options include the administration of adjuvants such as levamisole, an anti helminthic drug which has a property to stimulate depressed T-cell activity and enhance B lymphocyte function. Thus, levamisole has been used for up-regulation of defective immune

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3 function in patients with chronic renal insufficiency. The aim of this study was to evaluate the
4 available evidence on efficacy and safety of levamisole- adjuvanted HB vaccine in chronic dialysis
5 patients by performing a systematic review of the available literature with a meta-analysis of
6 randomised, clinical trials (RCTs).
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PATIENTS AND METHODS

Search strategy and data extraction

Electronic searches of the National Library of Medicine MEDLINE database, Current Contents and manual searches of selected specialty journals were performed to identify all pertinent literature. It had been previously demonstrated that an electronic search alone may not sensitive enough [19]. Four MEDLINE database engines (Ovid, PubMed, Embase, and GratefulMed) were used. The key words ‘hepatitis B vaccine’, ‘levamisole’, ‘vaccine adjuvant’, and ‘dialysis’ were used. Reference lists from qualitative topic reviews, and published clinical trials were also searched. Our search included human studies that were published in the English and non-English literature. All articles were identified by a search from January 1985 to June 2010. Data extraction was conducted independently by two investigators (F.F., V.D) and consensus was achieved for all data. Studies were compared to eliminate duplicate reports for the same patients, which included the contact with investigators when necessary. Eligibility and exclusion criteria were pre-specified.

Criteria for inclusion

To be included in this systematic review, a clinical trial had to fulfil a set of criteria. It had to be published in a peer-reviewed journal; only prospective, randomized controlled clinical trials comparing the response rate with levamisole plus HB vaccine (study group) versus HB vaccine alone (control group) were included. Studies which enrolled dialysis patients or patients with chronic renal failure not yet requiring the dialysis were eligible. Patients on chronic HD or peritoneal dialysis were considered for the current meta-analysis. Trials based on the plasma-derived or recombinant DNA hepatitis B vaccine were included. The decision as to inclusion or exclusion of clinical trials was not related to results. Patients who underwent primary vaccination

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3 schedule (naïve patients) or those who had failed to respond to prior vaccine schedule (non-
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5 responder patients) against hepatitis B were enrolled. All dose schedules and routes of vaccine
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7 administration were considered.
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10 11 12 13 14 15 Ineligible studies

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17 Studies were excluded if they reported inadequate data on measures of response. Clinical
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19 trials including hepatitis B surface antigen (HBsAg) positive patients or patients receiving
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21 immunosuppressive drugs, or with malignant diseases and human immunodeficiency virus infection
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23 were not considered. Trials that were only published as abstracts, or as interim reports were
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25 excluded; reviews articles were not considered for this analysis. Clinical trials that involved the
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27 renal transplant recipients were not considered.
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36 End-points of interest

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38 We evaluated the serological response to HBV vaccine in study and control groups. We
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40 compared the rate of patients with protective anti-HBs titers after the completion of HBV
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42 vaccination in study (HBV vaccine plus levamisole) vs. control (HBV vaccine alone) patients. The
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44 level of antibody production that defines immunity was 10 IU/mL across the trials. These
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46 definitions were consistent with the standards published in the scientific literature.
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55 Statistical methods

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57 Outcomes were analysed on an intention-to-treat basis, i.e., all patients included in these
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59 studies were considered for the calculation of the response rate, while patients without the end-point
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were considered as failures. The odds ratios (OR) for seroresponse after the vaccination (vaccine

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3 plus levamisole versus vaccine alone) were generated by using the random effects model. The
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5 random effects approach was made according to DerSimonian and Laird [20]. The Cochrane's Q -
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7 test was used for quantifying the heterogeneity [21]; I^2 was also used to provide a measure of the
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9 degree of inconsistency in the studies' results [22]. The Galbraith plot was used to assess the
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11 heterogeneity and precision of single studies [23]. Pooled ORs were calculated in the subgroups of
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13 clinical trials as sensitivity analyses; sensitivity analysis using a fixed-effect model was also
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15 performed to assess the consistency of results. The publication bias assessment (number of void or
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17 negative trials necessary to render the meta-analysis meaningless) was calculated according to the
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19 Klein formula [24]. The publication bias was also measured by the test of funnel plot asymmetry.
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21 The 5% significance level was used for alpha risk. Every estimate was given with its 95%
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23 confidence intervals (95% CI). In this study, the statistical software (RevMan 4.1; Update Software
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25 Ltd, Oxford, UK) freely provided by the Cochrane Collaboration was used.
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RESULTS

Literature review

Our electronic and manual searches identified 24 papers that were selected for full text review; the complete list of these reports is available on request. All candidate studies were English language publications. Twenty papers were excluded because they did not fulfil the inclusion criteria. As shown in Table 1, we identified four papers providing the information on four prospective, controlled clinical trials [25-28]. A total of 328 unique patients were included: 128 and 200 individuals in study and control groups, respectively. Three (75%) were randomized controlled clinical trials (RCTs); the randomization methods were adequately stated only in the study from Sali *et al.* [28] (a double-blind, placebo-controlled study; randomization was made by applying a table of random numbers). There was 100% concordance between reviewers with respect to final inclusion and exclusion of studies reviewed based on the predefined inclusion and exclusion criteria.

Patient characteristics

Shown in Tables 1-2 are some salient demographic and clinical characteristics of subjects enrolled in the included clinical trials. All trials were published from 2000 to 2008; two were from Turkey and two from Iran. All patients included in this study had end-stage renal disease; chronic hemodialysis patients underwent standard techniques (bicarbonate dialysis, three times weekly, 3 or 4 hours each session). Mean age of subject cohorts ranged from 41 ± 2 to 53 ± 17 years of age, and gender distribution ranged from 47% to 66% male (Table 2). The mean time on dialysis prior to initiation of treatment varied from 11 ± 1 to 53 ± 3 months, although one study did not report data on this characteristic (Table 2). As reported in Table 2, some studies concerned the re-vaccination (i.e., vaccination in dialysis patients who had failed to respond to prior IM vaccination). Data on vaccine schedule in the trials included in this meta-analysis are shown in Table 3. Recombinant HB vaccine was used in all studies. Eleven (8.6%) patients received HB vaccine by intradermal (ID) route among study, and 11 (5.5%) among control patients.

Summary estimates of outcome and side-effects

A significant increase in response rates among study (levamisole plus hepatitis B virus vaccine) vs. control (hepatitis B virus vaccine alone) patients was found (Table 4); the pooled Odds Ratio was 2.432 (95% Confidence Intervals, 1.34; 4.403), $P=0.002$ (random effects model). The test for heterogeneity was not significant [$Q=3.757$ ($P=0.289$), $I^2=46.8\%$]. The test of funnel plot asymmetry was not significant [$\alpha=0.22$; 95% CI, -6.78; 7.22 ($P=0.95$)]. The publication bias assessment (according to Klein formula) was 8.

The comparison between study and control groups at completion of vaccine course has been shown in the Galbraith plot (Figure 1); it reports the effect of treatment, the precision and the effect

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of each single trial included in the meta-analysis. It also provides information on the heterogeneity between trials.

As listed in Table 4, subgroup analyses were undertaken to explain the heterogeneity across trials. The pooled OR (according to DerSimonian and Laird) was 2.085 (95% CI, 1.193; 3.646) in the subgroup of dialysis patients receiving HB vaccine by IM route. The tests for study heterogeneity were $Q=1.755$ ($P=0.416$), $I^2=43.0\%$. The pooled OR was 2.184 (95% CI, 1.0; 4.72) in naïve patients with chronic uraemia; the heterogeneity test was $Q=4.793$, $P=0.188$, $I^2=58.3\%$.

In five (3.9%) patients adverse effects of levamisole were established. The side-effects were as follows: reversible mild leukopenia ($n=1$), non-specific dermatitis ($n=2$), mild generalized pruritus ($n=1$), and mild abdominal pain ($n=1$).

DISCUSSION

The impaired response rate to hepatitis B virus vaccine among patients receiving long-term dialysis has been addressed by several investigators. Alternative schedules with adjuvants have been reported; erythropoietin (EPO) [13], zinc, thymopentin [29], gamma-interferon [30], interleukin-2 (IL-2), granulocyte macrophage-colony stimulating factor (GM-CSF) [31], or additional immune modulators (AM3, AS04) [32-33] have been recently used in combination with hepatitis B virus vaccine in order to increase the rate of response in dialysis patients. The efficacy of these various approaches remains unclear.

Some investigators have recently suggested levamisole as an adjunct to increase response to HBV vaccination. The availability and low cost of levamisole supported this approach. Although levamisole has been introduced first for parasitic infections it also has immune modulating properties. It has been reported that levamisole stimulates T cells or macrophages; in addition, it restores delayed hypersensitive reactions in immune depressed patients [34]. Recent studies have reported that levamisole increases serum levels of IL-2 receptors in patients with cancer [35] and IL-1 production in mice models [36]. Levamisole is metabolized by the liver and then secreted from the kidney; no clearance by hemodialysis or peritoneal dialysis has been demonstrated. Some evidence on the efficacy of levamisole-adjuvanted HB vaccine in dialysis population already exists although only a few clinical trials with small size have been conducted.

We have reviewed the available evidence on efficacy and safety of levamisole as an adjuvant to HB vaccination in the dialysis population by a meta-analysis of randomized clinical trials (RCTs). We showed efficacy for levamisole administration in this setting; in fact, the pooled OR for seroresponse was significantly higher in study subjects (levamisole plus vaccine) versus control (vaccine alone) patients [OR, 2.432 (95% Confidence Intervals, 1.34; 4.403)]; no study

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3 heterogeneity was found. Stratified analysis demonstrated that our findings were robust; indeed, the
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5 analysis in various subgroups yielded only minimal changes on the effect size.
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8 This meta-analysis is subject to several limitations, an examination of which may inform the
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10 design and conduct of future studies on this topic. First, despite inclusion criteria that were more
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12 stringent than those of previous reviews, the methodological quality of the studies was on average
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14 not ideal. Among the four studies included in this review, only one described methods of
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16 randomization; it is possible that some so called 'randomized controlled trials' were not real
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18 randomized controlled trials owing to a lack of rigorous clinical trial design [37]. It has been
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20 already emphasized that trials with low methodology quality can increase the estimates of
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22 intervention efficacy reported in meta-analysis [38]. Second, a limited number of small-sized
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24 reports was available for this analysis even if none of the studies we included was published as an
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26 interim report. The information published in preliminary format (i.e., abstract) can suggest greater
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28 treatment effect [39]. We have not included cohort trials to increase the sample size of our analysis,
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30 as such studies have been shown to produce an exaggerated treatment effect that is probably biased
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32 [40]. Finally, data on seroresponse rate over follow-up were limited in our systematic review
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34 although this point is crucial as anti-HBs protective titers in chronic uraemia are lower and decline
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36 over time faster than among individuals with normal kidney function.
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43 An additional limitation of this study is the use of anti-HBs titer as a surrogate marker of
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45 protection against HBV infection. The protective effect of recombinant vaccine against acquisition
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47 of HBV infection among patients on long-term dialysis has been already reported by the CDC [41].
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49 The efficacy of levamisole plus vaccine versus vaccine alone against HBV should be evaluated in
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51 terms of reduced incidence of HBV infection in dialysis populations. The very low incidence of
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53 HBV infection among patients on maintenance dialysis in developed world clearly makes difficult
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55 the implementation of such clinical trials.
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59 In summary, this meta-analysis of RCTs has shown significant benefit of levamisole
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administration as adjuvant in order to increase the seroresponse rate to HBV vaccine in patients on

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3 long-term dialysis. However, the preliminary nature of these results cannot be underestimated.
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5 Large sized randomized clinical trials to confirm these findings should be considered.
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| Authors | Patients, <i>n</i> (study group) | Patients, <i>n</i> (control group) | Reference year | Country |
|--------------------------|-------------------------------------|--|-------------------|---------|
| Ayli MD, <i>et al.</i> | 30 | 30 | 2000 | Turkey |
| Kayatas M, <i>et al.</i> | 38 | 116 | 2002 | Turkey |
| Argani H, <i>et al.</i> | 22 | 22 | 2006 | Iran |
| Sali S, <i>et al.</i> | 38 | 32 | 2008 | Iran |

Table 1.

Baseline characteristics of studies included in the meta-analysis

Figures are given for study patients/ controls wherever appropriate

| Authors | Time on dialysis, mo | Age, years | Male (%) | Prior vaccine use |
|--------------------------|---|---|-----------------------|-------------------|
| Ayli MD, <i>et al.</i> | 11.8 \pm 1.1 | 41.4 \pm 2.4 | 34 (57%) | No |
| Kayatas M, <i>et al.</i> | 46 \pm 40/ 51 \pm 38/ 31 \pm 27/ 41 \pm 31 | 46.6 \pm 2/ 42.9 \pm 2 | 64 (55%)/ 23 (60%) | No/No/ Yes/Yes |
| Argani H, <i>et al.</i> | NR | 47 \pm 9/ 45 \pm 9/ 48 \pm 8/ 41 \pm 7 | 13 (59%)/ 12 (50%) | No |
| Sali S, <i>et al.</i> | 17.0 \pm 3.3/ 13.2 \pm 3.0 | 42.9 \pm 2.1/ 46.6 \pm 2.3 | 20 (53%)/ 21 (66%) | No |

Table 2.

Baseline characteristics of studies included in the meta-analysis

Figures are given for study patients/ controls wherever appropriate

NR= not reported

| Authors | Vaccine, schedule | Levamisole, schedule |
|--------------------------|--|-------------------------------|
| Ayli MD, <i>et al.</i> | 20 mcg IM x4 (0, 1, 6, 9 mo) (n=30) 40 mcg IM x4 (0, 1, 6, 9 mo) (n=30) | 120 mg per os x3/ week (9 mo) |
| Kayatas M, <i>et al.</i> | 40 mcg IM x3 (0, 1, 6 mo) | 80 mg per os x3/ week (4 mo) |
| Argani H, <i>et al.</i> | 40 mcg IM x1 20 mcg ID x1 | 100 mg per os daily (12 days) |
| Sali S, <i>et al.</i> | 40 mcg IM x3 (0, 1, 6 mo) | 100 mg per os x3/ week (6 mo) |

Table 3.

Vaccine and adjuvant schedules of studies included in the analysis

IM=intramuscular administration of vaccine

ID=intradermal administration of vaccine

| | Random-effects model OR (95% CI) | <i>Q</i> (<i>P</i>) | <i>I</i> ² |
|---------------------------------------|-------------------------------------|-------------------------|-----------------------|
| All studies (<i>n</i> =328 pts) | 2.432 (1.34 - 4.403) | <i>Q</i> =3.757 (0.289) | 46.8 |
| *IM patients (<i>n</i> =284 pts) | 2.085 (1.193 - 3.646) | <i>Q</i> =1.755 (0.416) | 43.0 |
| **Naive patients (<i>n</i> =251 pts) | 2.184 (1.004 - 4.72) | <i>Q</i> =4.793 (0.188) | 58.3 |
| European studies (<i>n</i> =214 pts) | 2.567 (1.36 - 4.848) | <i>Q</i> =0.064 (0.800) | 0.0 |
| ***Full course (<i>n</i> =284 pts) | 2.085 (1.193 - 3.646) | <i>Q</i> =1.755 (0.416) | 43.0 |

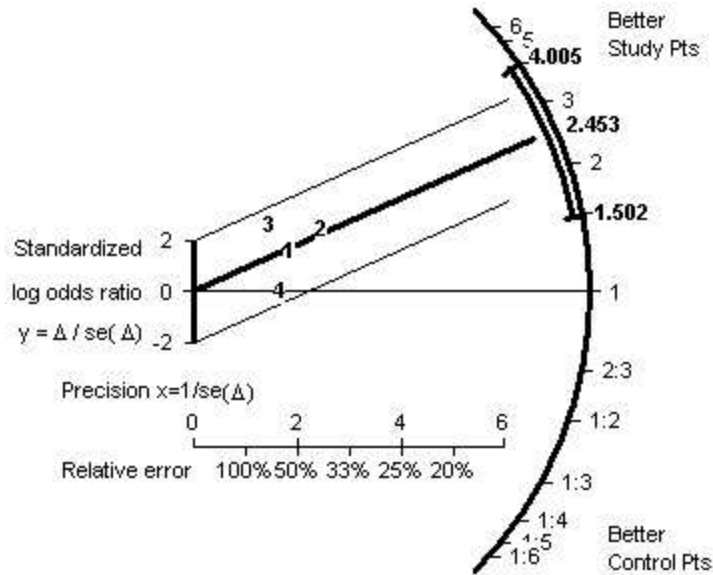
Table 4.

Pooled Odds Ratio (OR) of failure to respond to vaccine (study vs. control groups) in various subgroups of interest

*Only patients receiving HB vaccine by intramuscular route were included in the analysis

** Only patients who had never received HB vaccine prior the study were included in the analysis

***Only patients who received a full course of HB vaccine were included in the analysis



Levamisole as adjuvant to HB vaccine in dialysis patients: Galbraith plot

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