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Strategy for hepatitis A seroprevalence survey in a population of young people

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ABSTRACT

In the present research a novel operative strategy of health surveillance with a reduced number of serologic tests is proposed. The approach consists to identify sub-populations with high predictable serological profile that makes the serological tests unnecessary. The study is focused on assays done to detect the response against hepatitis A, which in Italy displays low/intermediate endemicity. Receiver operating characteristics analysis performed on data from documented and self-reported vaccination information of a cohort of students from Padua University Medical School confirmed that anti-hepatitis A antibodies measurement might be avoided in subjects younger than 30 years with negative documented or self-reported history of vaccination or subjected to current vaccination schedule.

1. Introduction

The present research aimed to build up a new operative strategy of health surveillance with a reduced panel of serological test and therefore with lower economic impact. The approach consisted in the identification of sub-populations with high predictable immunological profile in which the serological tests resulted unnecessary. We focused on the serological tests performed to detect the response against hepatitis A virus (HAV) infection. HAV is a mild disease frequently elapsing without symptoms, caused by ingestion of food and/or water contaminated by faecal materials. Improvement in hygiene and sanitation paralleled with economic and social advancement resulted in a dramatic decline of HAV circulation. Consistently, an Italian study performed on a cohort of military recruits [1] showed a relevant drop in prevalence of positive antibodies from 66.3% in 1981, to 29.4% in 1990, and to 5.3% in 2003. In the Italian population, last available data (2006) indicate that the incidence of the disease is 1.4/100 000 with a case-fatality rate of 2.9/10 000 [2]. Italy is a low/intermediate endemicity country [2] and the majority of HAV infections is caused by shellfish consumption and travel to endemic areas. The first statement could justify the significant prevalence of positive antibodies in population living in Southern Italy where consumption of raw shellfish is more common [3]. Together the decline of the disease due to increased hygienic behaviour and the following drop of immunisation favoured outbreaks of HAV infection as observed in Southern Italy during the years 1996–1998 [4,5], as well in Spain [6,7], Sweden [8], England [9], and Finland [10].

The development of safe and effective vaccine against HAV have been licensed since 1992 (HAVRIX®, GlaxoSmithKline) and the vaccination schedule (not mandatory) requires an i.m. injection at time 0 and after 6–12 months that guaranties persistence of the protection for a period longer than 10 years [11,12]. The availability of this vaccination leads to a further decrease of the frequency of the hepatitis A [13]. Accordingly, our previous study performed on subjects of the Padua University personnel revealed age-dependent seropositivity for HAV [14].

In the health surveillance program of University students provided according to Italian law for safety and health, the risk to catch an infectious disease has to be evaluated. Therefore, from each student of Padua University Medical School, infectious diseases, HAV included, and vaccination histories have been collected and the serology has been investigated.

This documented and self-reported information of a large cohort of students has been processed by means of the receiver operating characteristics (ROC) analysis in order to identify a subset of individuals with high predictable immunological profile.

ROC analysis has been at beginning used in signal detection theory [15] and then extended to diagnostic systems and clinical decision analysis [16,17]. In this study, ROC method has been validated and used to define new guidelines in the health survey, thus paying the way to further applications in public health control strategies.

2. Materials and methods

2.1. Participants

Since 2004–2010, 4928 students belonging to Padua University Medical School (medicine and surgery, dentistry, and health pro-

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fessions) have been subjected to health surveillance according to the law. The research is based on data gathered during the health surveillance, then an evaluation by ethic committee is not required.

All individuals have been submitted to fill up a questionnaire on HAV disease and vaccination. In the first round of analysis, 1687 subjects have been discharged because 1119 did not reply to the question on vaccination, 3 declared clinical disease (confirmed by positive antibodies), 364 originated from high endemicity countries (Eastern Europe, Africa, Asia and Central/Southern America), and 201 were older than 30 years. The reason of the exclusion of data regarding these last two groups relies on the fact that these individuals display high percentage of seropositivity that may affect the analyses.

The remaining 3241 individuals have been enrolled (2330 females and 911 males). Among the enrolled subjects, 1918 (1379 females and 539 males) provided a booklet where the vaccination history has been certified by the Public Health Office (164 of these, 113 females and 51 males, have been vaccinated against HAV), whereas 1754 (1266 females and 488 males) had not a vaccination document. Mean age of the enrolled subjects has been similar (22.1 ± 2.8 documented and 21.0 ± 3.1 not documented) with slight but significant ($p < 0.001$) differences according to gender (males older than females).

2.2. Anti-HAV antibody measurement

Anti-HAV (IgG and IgM) antibodies have been measured by means of a chemiluminescent microparticle immunoassay (CMIA) qualitative method (ARCHITECT®) by Abbott Diagnostic Division (Wiesbaden, Germany). The test is quantitative, then the results have been supplied as positive or negative.

2.3. ROC analysis

Analysis has been performed separately for documented and self-reported history of vaccination, and according to gender.

In a 2×2 contingency table (confusion matrix) vaccination history (positive or negative) and antibodies (positive or negative) have been matched. For every category (documented and self-reported, males and females) the outcome is a number of true positive (TP), i.e. positive vaccination history and antibodies, true negative (TN), i.e. negative vaccination history and antibodies, false positive (FP), i.e. positive vaccination history and negative antibodies, and false negative (FN), i.e. negative vaccination history and positive antibodies. As in Section 2.1, subjects declaring clinical disease have been excluded by the casuistry. Tables display: (1) TP rate (also sensitivity or recall), ratio between TP and total positive (TP + FN); (2) FP rate, ratio between FP and total negative (TN + FP); (3) accuracy, ratio between the sum of TP and TN on total subjects; (4) specificity, ratio between TN and the sum of FP and TN (all subjects with negative antibodies); (5) positive predictive value (PPV, also precision), ratio between TP and the sum of TP and FP (all vaccinated subjects); (6) negative predictive value (NPV), ratio between TN and the sum of TN and FN (all not vaccinated subjects); (7) false discovery (FD) rate, ratio between FP and the sum of FP and TP (all vaccinated subjects); (8) *F*1 score that means the test accuracy and depends on precision and recall (sensitivity) as follows: 2/(1/precision + 1/recall); and (9) Matthews correlation coefficient (MCC) generally used in machine learning as a measure of the quality of binary (two-class) classifications. It is generally regarded as a correlation coefficient between the observed and predicted binary classifications and it is calculated as follows: $\mathrm{TP} \times \mathrm{TN} - \mathrm{FP} \times \mathrm{FN} / \sqrt{(\mathrm{TP}+\mathrm{FP})(\mathrm{TP}+\mathrm{FN})(\mathrm{TN}+\mathrm{FP})(\mathrm{TN}+\mathrm{FN})}$ [18].

Ninety-five percent confidence interval (95% C.I.) has been finally determined for PPV and NPV. Other statistical analyses have been performed by means of χ^2 (two-tailed test) with Yeats correction; significance has been stated from $p < 0.05$. Statsdirect 2.7.7 version (Statsdirect Ltd., UK) has been used for statistical analyses.

Table 1
ROC analysis applied to documented students (30 or less year old) belonging to Padua University Medical School since 2004–2010.

	All subjects	Males	Females
No.	1918	539	1379
TP rate	0.899	0.935	0.885
FP rate	0.007	0.016	0.004
Accuracy	0.984	0.980	0.986
Specificity	0.993	0.984	0.996
PPV	0.921	0.843	0.956
95% C.I.	0.880–0.962	0.743–0.943	0.918–0.994
NPV	0.990	0.994	0.989
95% C.I.	0.985–0.995	0.988–1.000	0.983–0.995
FDR	0.079	0.157	0.044
*F*1 score	0.910	0.887	0.919
MCC	0.901	0.877	0.912

P: positive; N: negative; TP: true positive; FP: false positive; TN: true negative; FN: false negative; PPV: positive predictive value; NPV: negative predictive value; FDR: false discovery rate; MCC: Matthews correlation coefficient; TP rate: TP/P (TP + FN); FP rate: FP/N (FP + TN); accuracy: (TP + TN)/(P + N); specificity: TN/(FP + TN); PPV: TP/(TP + FP); NPV: TN/(TN + FN); FDR: FP/(FP + TP); $\mathrm{MCC} = (\mathrm{TP} \times \mathrm{TN} - \mathrm{FP} \times \mathrm{FN}) / \sqrt{(\mathrm{TP}+\mathrm{FP})(\mathrm{TP}+\mathrm{FN})(\mathrm{TN}+\mathrm{FP})(\mathrm{TN}+\mathrm{FN})}$; *F*1 score: 2/(1/PPV + 1/TP rate). 95% C.I. has been determined for PPV and NPV only.

3. Results

3.1. Analysis of the prevalence of anti-HAV antibodies according to geographical origin

Individuals originated from high endemicity countries showed high percentage of seropositivity that might affect our analysis. Therefore data regarding this group of students have been discharged. Fig. 1 shows the prevalence of positive anti-HAV antibodies according to geographical origin of the students. The analysis of this figure confirms the expected high rate of positive anti-HAV antibodies in African students (92.2%), but also in those from Eastern Europe, Asia and Central/Southern America [19], and supports the requirement of the exclusion of these students from our casuistry. In particular, the results regarding Albanian students confirmed those of previous research [20] showing 84.6% of positive anti-HAV antibodies.

In addition, a significantly higher prevalence ($\chi^2 = 65.312$, $p < 0.001$) of positive antibodies has been observed in students from Southern compared to those from Northern/Central Italy. Western Europe has not been represented due to the small size of the sample.

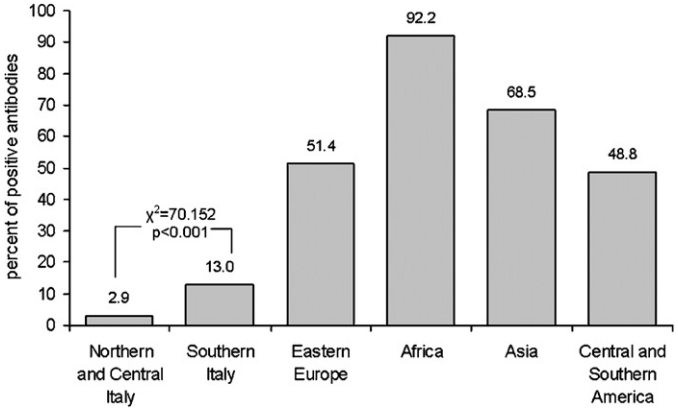

Fig. 1. Seroprevalence of HAV antibodies according to geographical origin of the students.

Table 2
ROC analysis applied to not documented students (30 or less year old) belonging to Padua University Medical School since 2004–2010.

	All subjects	Males	Females
No.	1323	372	951
TP rate	0.500	0.440	0.521
FP rate	0.035	0.058	0.026
Accuracy	0.931	0.909	0.940
Specificity	0.965	0.942	0.974
PPV	0.527	0.355	0.617
95% C.I.	0.424–0.630	0.186–0.524	0.494–0.740
NPV	0.961	0.959	0.962
95% C.I.	0.950–0.972	0.938–0.980	0.949–0.975
FDR	0.473	0.645	0.383
*F*1 score	0.513	0.393	0.563
MCC	0.477	0.346	0.535

For legend see Table 1.

3.2. Validation of the ROC analysis and identification of two subsets of individuals with high predictable serological profile

Up to now, ROC analysis has been used to assess sensitivity, specificity and accuracy of diagnostic tests. In order to ascertain whether the method is useful for our scope, we have set up a comparative analysis between documented vaccination and seropositivity. The readout strategy has been the correlation seropositivity/vaccination (after at least two doses) and seronegativity/absence of vaccination.

Among the 1918 individuals providing documented vaccination 164 have been vaccinated against HAV (8.6%): 79 received one dose of vaccine, 66 two doses and 19 three doses. Among these 164 subjects, 13 showed negative antibodies (7.9%); however the majority of individuals showing negative anti-HAV antibodies (11 on 13) has been subjected to one dose of vaccine, one individual has been vaccinated twice and one three times. Thus, only two cases presenting negative antibodies (2.4%) have been found among the individuals vaccinated according to vaccination schedule (two or more doses, 85 individuals).

As new findings, the probability to detect negative antibodies after vaccine has been significantly higher ($\chi^2 = 4.661$, $p < 0.05$) in males (15.7%) than in females (4.6%), independently of the vaccination schedule.

Table 1 reports values obtained by ROC analysis: high positive (PPV, 0.921) and negative predictive values (NPV, 0.990) as well high accuracy (0.984), and specificity (0.993) are really promising. Among the subjects lacking of vaccination (1754), the rate of positive markers has been found very low (1.0%), without difference according to gender.

Taken together, these results suggest that ROC analysis is instrumental for the study and outlines two sub-populations of students with high predictive serological profile.

3.3. Reliability of self-reported history of vaccination and identification of an additional subset of individuals with high predictive seronegativity

The next issue addressed has been the reliability of self-reported information, representing a conspicuous piece of data. Among the 3241 students of our cohort, 1323 without booklet provided information about their vaccination history filling up a proper questionnaire. The analysis of these data, illustrated in Table 2, revealed low PPV (0.527), high NPV (0.961), very good accuracy (0.931) and specificity (0.965). A more detailed analysis revealed that the probability to find negative antibodies appeared higher ($\chi^2 = 50.543$, $p < 0.001$) in individuals self-reporting vaccination (47.3%) than in subjects providing documentation (7.9%). By contrast, the probability to find positive antibodies has been found significantly higher ($\chi^2 = 27.755$, $p < 0.001$) in individuals self-reporting no vaccination (3.9%) than in subjects documenting absence of vaccination (1.0%). Curiously, females self-reporting vaccination have been significantly more reliable ($\chi^2 = 4.620$, $p < 0.05$) than self-reporting males. Any differences related to gender have been found in self-reported absence of vaccination.

4. Discussion

Health survey of University workers and students requires a large panel of serological tests implying high cost for the academic administration and concern for single individuals.

In the present research, the assessment of a novel approach to the health surveillance with the aim to reduce the number of serological tests has been proposed. This issue has been tackled focusing on assays done to detect the response against mild infective diseases such as HAV. In fact, HAV displays low/intermediate endemicity in Italy due to the improvement of hygienic conditions and outbreaks are localized to specific areas. In addition, since 1992 vaccination is available. For these reasons, the seropositivity of hepatitis A is directly age-related, in other words middle-age and old people more probably have contracted the infection whereas young people resulted protected. Consequently, the here analysed cohort of students is expected to be seronegative.

The working approach has been to identify a sub-population of individuals with high predictable serological profile making the serological test unnecessary.

Documented and self-reported sanitary information has been collected in a cohort of students from Padua University Medical School and then processed by means of the ROC analysis. In order to test the applicability of the ROC analysis, the predictive values of documented vaccination/absence of vaccination related to seropositivity/seronegativity have been first investigated. The literature reports that the vaccination efficacy relies between 94% and 100% [21,22], although the vaccine appears less immunogenic in chronic liver diseases [23], in transplant recipients [24], and in the elderly [25]. Being recommended almost two-dose schedule, the here obtained results are consistent with previous investigations [22,23]: from the ROC analysis came out the expected high predictivity of seropositivity after vaccination, thus confirming its applicability to our scope. Two subsets of individuals not requiring anti-HAV antibody detection have been then identified, represented by those providing documented vaccination according to the schedule and others giving documentation of absence of vaccination.

Among 164 vaccinated individuals, 85 received at least two doses, according to the schedule (time 0 and after 6–12 months). Ten individuals have been vaccinated twice with an interval between the two doses larger than 12 months (mean value 29.4 months) and notwithstanding this, the following survey still showed effective protection against HAV. These results, even if collected from a small sample, suggest that a new interval between the administration of the two doses of vaccine might be considered, opening the way to further investigations.

ROC analysis has been then applied for the evaluation of data obtained also from a self-reported history of vaccination. The results showed high accuracy (0.931) and specificity (0.965), and high NPV (0.961), similar to that determined in documented subjects. By contrast, the PPV has been found low and not consistent with the PPV found in the analysis of documented data. These findings clearly indicate that a negative reply to the question on vaccination matches with the discovery of negative antibodies, accounting for the suppression of the serological analysis.

These results suggest that through the ROC analysis is possible to identify subsets of individual characterized by high PPV and NPV

from documented vaccination histories and high NPV from self-reported information. Establishing a really high cut-off of 0.90, two groups have been identified: (1) subjects younger than 30 years old, originated from Northern/Central Italy with negative documented and self-reported history of vaccination and (2) subjects providing booklet of vaccination submitted to current vaccination schedule (two doses).

The new strategy foresees to spare these individuals from the blood sample analysis of anti-HAV serology since they display high predictable serological profile. In Veneto Region, the cost of anti-HAV IgG measurement corresponds to € 12.10 per test: testing all 3241 individuals of our cohort would cost € 39,216.10. The findings suggest that serologic test might be unnecessary in individuals submitted to two or more doses of vaccine (85 subjects, saving € 1,028.50) and in individuals documenting absence of vaccination (1754 subjects, saving € 21,223.40) or self-reporting absence of it (1232 subjects, saving € 14,907.20). The final cost of serologic test would be lowered to € 2,057.00.

Taken together the present data point out that ROC analysis is useful to evaluate self-reported history of vaccination against diseases with low endemicity, low diffusion among population, infrequent and well circumscribed outbreaks. By contrast, the analysis is inappropriate for the evaluation of self-reported history of mandatory vaccination due to the expected high prevalence of positivity. For instance, as demonstrated by our previous results [26], vaccination against hepatitis B displays high PPV because is mandatory in Italy since 1991. Similarly, the so-called exanthematic childhood diseases such as rubella, mumps and measles usually show a high rate of positive antibodies related not only to vaccination (not mandatory in Italy) but also to the history of clinical disease [26,27].

Finally, PPV determined in self-reported history of vaccination against HAV is low; a possible explanation could rely in misunderstanding the question on HAV vaccination, frequently confused with that on hepatitis B. In addition, females appear more reliable than males when solicited to remember vaccination with a larger correspondence between positive history and discovery of seropositivity.

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