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SPf66 VACCINE TRIAL IN BRAZIL: CONCEPTUAL FRAMEWORK STUDY DESIGN AND ANALYTICAL APPROACH

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This paper describes the study population and the study design of the phase III field trial of the SPf66 vaccine in Brazil. Assessment of validity and precision principles necessary for the appropriate evaluation of the protective effect of the vaccine are discussed, as well as the results of the preliminary analyses of the gathered data. The analytical approach for the estimation of the protective effect of the vaccine is presented. This paper provides the conceptual framework for future publications.

Key-words: Malaria vaccine. SPf66 vaccine trial. Vaccine efficacy.

The main objective of a vaccine phase III trial is to estimate the direct protective effect of the vaccine in the individual (vaccine efficacy) or, in other words, the alteration of the vaccinated individual's susceptibility to infection[4 22 26]. In order to obtain a valid and precise estimation of the efficacy of a vaccine that allows for a biological meaningful interpretation, several conditions must be satisfied in a field trial.

With the purpose of providing the conceptual framework of the phase III trial of SPf66 vaccine against the asexual blood-stages of *Plasmodium falciparum* in Brazil, we worked based upon the sequence of pathogenic processes that would lead to the endpoint(s) of interest the vaccine is suppose to prevent or modify as illustrated by Struchiner et al.[21]. Such framework divides the process in three sequences, each one with important issues of validity and precision principles: 1) selection of a susceptible study population, determination of an adequate sample size, definition and measurement of relevant factors (covariates), and the random vaccine allocation; 2) determination of the amount of exposure to infection the study population is subjected to; and 3) the definition of a response model.

Study area and population. The field trial was carried out in the rural settlements of the municipality of Costa Marques, Rondonia in the Amazon basin of Brazil. The region is characterized by a hot humid, equatorial climate, with seasonal distribution of rains (dry season from May to September and a rainy season from October to April). The vegetation is composed of dense tropical forest[9].

In the past 15 years, Costa Marques showed a significant increase of its population. In 1980, the municipality had a population of 2,998 inhabitants distributed in the urban (village of Costa Marques and Forte Principe da Beira), and rural areas (communities along the Guaporé river) each with 1,227 and 1,771 inhabitants, respectively[9]. Immigration of hundreds of people from different regions of the country took place after the opening of a road to the municipality (BR 429) in 1985, and the promotion of colonization by the Brazilian Government. The migrants settled mainly in the village of Costa Marques and along the new opened road. Using the information gathered by the National Health Foundation (FNS) on the number of inhabitants per house sprayed (coverage greater than 95%) in 1991, we estimated the urban population in approximately 14,445 inhabitants. Along the

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river, the approximate population size was 2,625, and along the road, the population was, 5,975 for a total of 23,045 inhabitants (FNS: unpublished data).

The number of cases reported by the FNS, showed a dramatic and progressive increase during the 80's, apparently related to the migratory influx. In 1983, 3,125 cases were reported in contrast to 16,029 cases in 1987. The dramatic increase in the number of cases and the progressive occupation of the forest, stimulated the establishment of specialized centers along the road where free diagnosis and treatment is offered to the population. Health agents were trained to refer to the main health center in the village all possible severe cases. Biannual spraying of all houses with insecticides of residual action and the treatment of presumptive cases detected by active search in the field were maintained. A reduction in the number of cases was then observed, probably due to the strengthening of the active and passive case detection procedures, as this control measure has an impact on transmission (7,532 cases were reported in 1990). Although malaria is considered the main health problem in the municipality, mortality is low probably due to the easy access by the population to diagnosis and specific treatment[24].

Malaria cases have a seasonal distribution, being higher during the beginning and the end of the rainy season. During the early colonization stage and before the strenghthening of control measures, the prevalence of malaria varied between 8 to 26%, according to the area. In the communities along the road the prevalence ranged between 14 to 26%, and in the village between 8 to 9%, being the ratio of *P. falciparum* and *P. vivax* infections 2:1. Spleen rates in children 2 to 10 years old ranged from 17% to 67% (depending on the year season), both in residents of the urban and rural area, a mesoendemic transmission level[15]. Cases were observed in all age groups and parasitemia was usually accompanied by the classical symptoms being subclinical infections, an exceptional finding[17]. The prevalence in infants was of 9% and the intensity of clinical manifestations had no apparent relationship with age. Malaria infections during pregnancy did not relate with parity. In addition, complications associated with parasitemia during gestation were rare, as well as congenital malaria, probably due to the frequent therapeutic use of antimalarials. IgG antibodies against asexual blood forms of *P. falciparum* were detected in 42% of the parturients at very low titers and showed a rapid decay[23]. In the last past years, *P. vivax* infections predominate and a reduction on prevalence was observed, achieving 10% in a preliminary survey for the vaccine trial done in residents along the road in 1990.

The FNS therapeutical schemes for treatment of malaria caused by *P. falciparum* have changed frequently during the past few years, due to the occurrence of multidrug resistance, as suggested by *in vivo* studies[16]. Chloroquine is used exclusively for the treatment of *P. vivax* infections, since sensitivity of *P. falciparum* to it was 18.2%. The sensitivity to sulfadoxine-pyrimethamine was 53.4%, to quinine of 81.8% and to quinine plus tetracycline of 93%. The present treatment scheme is based on the combination of quinine plus tetracycline in patients older than seven years of age and non-pregnant women. During pregnancy, the first line treatment is quinine in a 10 days schedule. Mefloquine is available and prescribed in case of resistance to the conventional scheme.

Anopheles darlingi is the main local vector of *P. falciparum* and *P. vivax* malaria, and corresponds to 91% of the thirteen species identified in Costa Marques[10 11 12]. Studies on the biting behavior showed that *A. darlingi* were active throughout the night with peak biting activities in the early morning and evening. Together with *A. deaneorum*, *A. darlingi* is the most anthropophilic and endophilic species of anophelines in the region[13]. Although they can be captured throughout the year, the population distribution of anophelines is closely related to the water levels of the Guaporé River and its tributaries, as well as with the beginning and ending of the rainy season. Upon closer examination of the data gathered in these surveys, the average biting rate per man per night in the near housing areas ranged from 53 to 301, depending on the year season, being lowest during the last trimester of the year, and highest on the second. Preliminary data suggested that the proportion of *P. falciparum* infected anophelines captured was 0,7%, as determined by ELISA. However, less than 50% of these anophelines had sporozoites in their

salivary glands (TA Klein: personal communication, 1989).

The population of interest for the field trial was that settled along the road, as it is composed mainly of migrants. After arriving to the area, the main activities of the immigrants were the building of temporary shelters, clearing the forest and, growing subsistence crops. They are very unstable, since their permanence depends mainly on economic success and health conditions. Once settled, the main occupations are agriculture and lumbering[15].

The clinical, serological and entomological data, as well as the population dynamics, characterize Costa Marques as an "agricultural frontier"[25] of medium endemicity, with periods of higher and lower transmission, occupied recently by non-immune individuals.

SPf66 vaccine. The synthetic vaccine SPf66 consisted of a sequence of three fragments derived from merozoite proteins linked by a sequence of the tetrapeptide of the circumsporozoite protein of *P. falciparum.* One milliliter of the product contained 4mg of the synthetic peptide solubilized in saline solution absorbed onto 2mg of aluminum hydroxide[1]. It was produced in the Instituto de Inmunologia, Universidad Nacional de Colombia by the research group of Dr. M.E. Patarroyo. The vaccine, as well as the placebo (tetanus toxoid for 1st and aluminum hydroxide for 2nd and 3rd doses), were bottled in clear glass recipients containing 10 doses each and coded with the letters *S* and *L* in Colombia. The vaccination schedule was defined as the subcutaneous application of 0.5ml on days 0, 30 and 180[18].

The possible direct effects of SPf66 malaria vaccine are the modification of clinical manifestations, including duration, since its main mechanism of action would be the induction of the production of specific antibodies against antigens of the asexual forms of *P. falciparum,* capable of limiting their multiplication[19]. The vaccine mechanism of action would mimic the acquisition of partial immunity observed naturally in highly endemic areas.

Incidence of clinical cases could be reduced by indirect means. The protective agent could interfere with the hepatic schizogony, preventing the development of parasitemia simulating the effect of a vaccine that prevents infection. If the protective effect of the vaccine is manifested by the presence of subclinical parasitemias, a reduction in the reported number of clinical cases will be noticed and/or, if the duration of parasitemia is diminished, there could be an effect on the production of the sexual forms altering transmission[7 20 26].

Study design. The objective of this trial was to evaluate the efficacy, immunogenicity and safety of the SPf66 vaccine in non-immune residents of a Brazilian endemic region. The main specific objectives were: first, to estimate the overall protective direct effect of the vaccine for all episodes, as well as crude and stratum specific estimates for the first and second episodes separately for *P. falciparum* and *P. vivax* infections. A futher objective was to detect possible differences in asexual blood-stage parasite densities. Second, to assess the immunogenicity of the vaccine and its relation with malaria infections, as well as its possible relation with protection, and third, to describe and determine the frequency of immediate or delayed side-effects associated with the application of SPf66 vaccine.

The study consisted of a randomized, double-blinded, placebo-controlled, efficacy trial. All volunteers, male or female, aging 7 to 60, resident in the rural settlements along km 52 to 112 of the road BR 429 were randomly assigned to a number between one to 800. Individuals suffering of acute or severe diseases, with history of allergies and pregnant women were excluded. All participants with an odd number received the preparation labeled *L* and those with an even number the preparation labeled *S*, shipped "ready for use", by the Colombian laboratory.

The required sample size was calculated according WHO's guidelines for phase III field trials[26]. Considering a prevalence of 10% and a vaccine efficacy of 80% (as established in the first field trial[1]), the sample size to detect such a difference at a 5% level of significance with 80% power was of 134 individuals in each group. Assuming a 30% loss to follow-up, the sample size increased to 179 subjects. Finally,

adding a design factor of 2, a total of 358 subjects in each treatment group seemed appropriate. We included 400 individuals in each group.

Study participants were registered with an individual identification number. The following information was recorded for each one: name, sex, age, site of residence, birth place, place of last residency, date of arrival to the study area, previous history of malaria episodes (number, usual symptoms and complications, date of last infection), and clinical history of allergies or other diseases.

Vaccine and placebo preparations were applied in three doses: the 1st on day 0, the 2nd on day 30 and the 3rd on day 180. The recommended dose of 0.5ml was applied subcutaneously in the deltoid region.. To facilitate the detection of local reactions, the first and third doses were applied on the right arm and the second, on the left.

Trial end-points: determination and measurement. Considering the objectives of the trial, several end-points were determined and measured: 1) incidence of malaria cases by species, 2) density of asexual blood-stage parasitemias, 3) determination of specific IgG antibodies against blood-stage antigens and vaccine peptides and, 4) frequency and description of side effects.

In order to determine the incidence of infection, two methods of evaluation were used: programmed periodic visits and passive and/or active search done by the FNS health agents. Periodical active searches were programmed on every vaccination (days 0, 30 and 180), and at regular intervals: every two months in the first year of follow-up and every three months in the second (Table 1). Blood samples were obtained for parasitological examination in the field. The administration of an effective schizonticidal drug to all participants before the beginning of the trial was not done. Shortly after the application of either dose of the vaccine or placebo, all participants with asexual parasitemia were adequately treated. The microscopists in charge of the diagnostic and treatment stations, as well as the field agents, were responsible for the notification of all cases of infection identified in the study subjects during the whole study period by passive or active search, specially during the intervals between the programmed active searches. The subjects'

Table 1 - Activity chart.

Day	Activity vaccination	Evaluation spleen	sero-parasitology	hct	leucogram
0	1st dose	x	x	x	x
30	2nd dose	x	x	x	x
45			x	x	
90			x	x	
180	3rd dose	x	x	x	
195			x	x	x
240			x	x	
300			x	x	
360			x	x	
450			x	x	
540	1st year	x	x	x	
630			x	x	
720		x	x	x	

3rd dose: August, 1991. End of follow-up: February, 1993.

name, register number, date and parasitological result were adequately recorded in specific notebooks. A written laboratory report was given to the participant. Treatment was supplied in case of the presence of asexual parasitemia Health agents were instructed to preserve all thick blood films for further blind re-examination at the reference laboratory.

Incidence of malaria infection was determined by the confirmation of the information obtained from each study subject by one of the following means: written parasitological reports given to the study subject, malaria infection records at the FNS stations, confirmation of asexual parasitemia by the revision of thick blood films in the reference laboratory and parasitological results obtained in the programmed active searches.

Malaria was diagnosed by the microscopic examination of thick blood films stained as recommended by the Pan American Health Organization[14]. All samples were examined by the FNS microscopist in the field. Negative results were determined after the observation of 200 microscopic fields. All samples (positive and negative) were preserved and sent to the Malaria Laboratory of the Núcleo de Medicina Tropical e Nutrição of the University of Brasília, where a blind re-examination was carried out for reliability of results. Asexual blood-stage densities were calculated by counting asexual forms per 500 white blood cells and expressed as number of parasites per mm^3. Evaluation of spleen size was performed in the prone position on every programmed vaccination, and on days 540 and 720.

Surveillance for mortality among study subjects was maintained. Information was

obtained from members of the community, hospital registers and/or the FNS records.

During the programmed active searches, capillary blood samples were collected from the study subjects by finger puncture into three heparinized microcapillary tubes. After centrifugation and hematocrit determination, plasma samples were adequately packed, stored and taken to the Laboratory of Malaria of the University of Brasília. IgG antibodies against asexual forms of *P. falciparum* were determined using the indirect immunofluorescence method, as well as antibodies against the vaccine peptides (ELISA) for evaluation of immunogenicity.

A surveillance system for detecting any adverse reaction was set up during every programmed vaccination. All participants were closely monitored during the first 30 minutes by the medical investigator in charge of the applications. Emergency equipment and therapeutic procedures were readily available. Inquiry for possible symptoms and identification of local reactions were performed two hours after each application, and four weeks after the 1st dose and two weeks after the 2nd and 3rd doses. Participants were advised to search for medical care in case of possible severe signs and symptoms secondary to vaccination. The frequency and intensity, as well as the description of any adverse effect, was systematically recorded.

Assessment of validity and precision principles. A preliminary step in the analyses, is the description and critical evaluation of the data gathered during the study. During this phase, the assessment of validity and precision principles is necessary. Once determined, it is possible to define relevant epidemiologic categories, select parameters, define statistical models and establish the methods most appropriate for the estimation of vaccine efficacy and/or other endpoints of interest[21].

The first happening that takes place in the process, is the selection of a susceptible study population. This is a straight forward condition for the evaluation of vaccine efficacy. The random allocation of the vaccine and placebo, as well as the "double-blindness", were used with the intention of homogeneously distributing known and unknown sources of heterogeneity among "treatment" groups. Known factors (covariates), which can help discriminate possible heterogeneities of exposure and/or susceptibility to infection among the study population, were identified and quantified during the study.

For determining the susceptibility to infection of the study population, information related with the number of previous malaria episodes, spleen size and presence of IgG antibodies against the asexual forms of *P. falciparum* was obtained prior to the beginning of the study. The presence of one or all of these variables, indirectly indicate that the participant was exposed, was capable to react inmunologically and was susceptible to infection. However, their absence does not necessarily imply resistance to infection (eventhough it is possible), since most of the individuals were migrants with different time since arrival to the endemic area.

Although we may assume an equal exposure to infection in both groups because of randomization, we can expect differences in the amount of exposure (heterogeneity) within each group. Transmission is also affected by seasonality, other concomitant control measures and by the vaccine itself, depending on the proportion of the population vaccinated (herd immunity). Baseline transmission levels prior to the intervention and during the study, can be approximated using information that indirectly denotes differences in exposure to infection. We considered age, sex, time since arrival to the endemic area, reported number of previous malaria episodes, spleen size, and antibodies titers at the beginning of follow-up. Antibody determination and quantification, is a reasonable indirect indicator not only for susceptibility, but also for exposure to infection. Differences in exposure to infection are also related to the number of malaria episodes, as well as age and sex. Splenomegaly has been considered an indicator for malaria endemicity and therefore an indicator for exposure to infection, however, its value as an indicator for exposure is questionable in areas where rapid diagnosis and specific treatment are available to the population, as part of the control programs. The periodic measurement of the considered factors allows to track changes in exposure to infection, as well as in susceptibility, which is altered by the vaccine.

Since the complete vaccination schedule of SPf66 vaccine consists in the application of three doses with a time interval of six months between the 1st and 3rd dose, several other

aspects must be taken into account: 1) the modification of the initial cohort characteristics up to the moment of completion of the vaccination schedule given the presence of time-dependent covariates, such as, the number of malaria episodes and humural immune response, and 2) losses to follow-up, which would constitute an important limitation that affects mainly the precision of the estimate of vaccine efficacy, making necessary the determination of its magnitude and its causes. Both conditions may lead to the loss of the desired effect of randomization (comparability of the treatment groups) with time.

Therefore, the preliminary analyses of data includes the description of the the study population with respect to the susceptibility/exposure factors previously discussed, assesment of randomization and definition of losses to follow-up.

Preliminary analyses of the data. The present study started in January 1991 and follow-up ended in February 1993. A total of 800 volunteers of both sexes aging 7 to 60 years, were randomly allocated to one of the treatment groups (400 participants on each group). Most of them were migrants (99.6%), natural of non-endemic areas of the country and residents in seven different localities of the rural area of Costa Marques.

Eventhough both treatment groups can be considered comparable at enrollment (Table 2), the data suggests that the population is heterogeneous within each group, not only with respect to age and sex, but also related to the previous contact with malaria infection. A simple correlation analysis revealed that the number of referred previous malaria episodes as well as the antibody titers, are positively correlated with age ($r = 0.16$ and $r = 0.19$, $p < 0.001$) and time since arrival to the endemic region ($r = 0.37$ and $r = 0.22$, $p < 0.001$). This suggests differences in exposure to infection among the participants. With respect to sex, males had in average more malaria episodes than females (4.6 and 3.1, respectively; $p = 0.002$). However, no differences in time since arrival and antibody titers were detected between sexes. The prevalence of malaria infection at the beginning of the trial was low (5.6%). *P. vivax* (4%) predominated over *P. falciparum* (2%) infections. The proportion of participants infected in both groups with either species was comparable. Classic symptoms related to malaria infection were referred by more than 95% of the participants in both groups.

Table 2 - Pre-vaccination cohort characteristics.

Characteristic	Vaccine		Placebo		p*
	n	%	n	%	
Age					
< 11	57	14	75	19	0.32
11 - 20	131	33	127	32	
21 - 50	185	46	168	42	
> 50	27	7	30	7	
Sex					
Male	247	62	245	61	0.88
Female	153	38	155	39	
Time since arrival (years)					
< 1	158	40	162	41	0.95
1 - 2	61	15	62	15	
2 - 3	66	16	60	15	
> 3	114	29	114	29	
Previous malaria episodes					
None	38	10	51	13	0.25
1 - 3	100	25	87	22	
several	262	65	262	65	
IFA antibodies					
positive	115	29	115	29	0.88
negative	277	71	279	71	
Spleen					
palpable	90	23	99	26	0.40
unpalpable	297	77	284	74	
Prevalence of infection					
P. falciparum	9	2	9	2	0.63
P. vivax	16	4	11	3	
negative	367	94	370	95	

*p-values for χ^2 tests for differences between groups

Prior to intervention, at least 89% of the participants had being exposed to infection (by either species) and, therefore, were susceptible to it. Only 29% had detectable antibodies against the assexual form of *P. falciparum* at very low titers (less than 160). After the completion of the vaccination schedule, 93% had at least one episode of malaria and 57% were seropositive.

The number of participants lost to follow-up before completing the vaccination schedule was considerable (28.5%). Of the initial cohort (800), 714 participants received the 2nd dose and 572 the 3rd. The most probable effects of losses are reducing the precision of the statistical estimate of vaccine efficacy and the introduction of selection bias[2]. Assessment of ramdoness of losses to follow-up, in the sense of being independent of the relationships under study[3], is essential for evaluating the possible presence of selection bias and for assessing the comparability of the treatment groups.

Losses to follow-up could be related with the disease (malaria episodes between the 1st and 3rd dose), and/or with secondary adverse effects of the vaccine/placebo applications during the 1st and 2nd doses. To assess these possible relationships, four variables were created based on information gathered during that period of time: number of times the participant had asexual parasitemia independent of the plasmodia species, number of times the individual searched for diagnosis, presence or not of secondary adverse reactions during the 1st and/or 2nd application of the vaccine/placebo, and time of survival until the application of the 3rd dose. Comparisons were made between survivors and lost to follow-up participants for each treatment group (Table 3).

Table 3 - Characteristics of lost to follow-up and survivors with respect to possible related variables.

Characteristic	Lost					Survivors				
	vaccine		placebo		p	vaccine		placebo		p
	n	%	n	%		n	%	n	%	
Parasitemia					0.8					0.6
never	54	48	61	53		135	47	145	51	
once	33	29	33	29		76	27	75	26	
twice	18	16	14	12		38	13	37	13	
> Twice	8	7	7	6		38	13	28	10	
Diagnoses search					0.3					0.9
never	35	31	49	42		85	30	85	30	
1 - 2	53	47	41	36		108	38	110	39	
3 - 5	16	14	16	14		73	25	69	24	
> 5	9	8	9	8		21	7	21	7	
Adverse reactions					0.3					<0.001
yes	23	20	17	15		105	37	33	12	
no	90	80	98	85		182	63	252	88	
Survival time (wks)					0.5					0.8
mean	12.5		11.5			26.5		26.5		
Total	113		115			287		285		0.9

* p value for χ^2 test for differences between groups and/or for comparison of means (t-student test).

The proportion of participants, as well as the average time of survival were comparable between both treatment groups in the participants lost to follow-up and survivors. No differences between the proportion of vaccinated and unvaccinated individuals were detected between lost to follow-up and survivors, in relation to the number of times they had detectable parasitemia or the number of times they searched for diagnosis (relation with disease). Eventhough the proportion of individuals with secondary adverse reactions was significantly greater in the SPf66 group, the proportion of individuals lost to follow-up who had secondary reactions was considered equal for both treatment groups. Comparability between lost and survivors was also maintained with respect to all other variables. Losses seem to be independent of the disease and of secondary adverse reactions.

The main causes of losses were absence of the participants and the presence of any acute disease during the programmed vaccinations, and emigration of the study area. Deaths were not related to malaria or treatment (Table 4).

Table 4 - Causes of losses to follow-up before the completion of the vaccination schedule.

Cause	Vaccine		Placebo		Total	
	n	%	n	%	n	%
Absence	59	52.2	58	50.4	117	51.3
Diseases	19	16.8	19	16.5	38	16.7
Emigration	16	14.1	20	17.4	36	15.8
Pregnancy	8	7.1	10	8.7	18	7.9
Withdrawal	8	7.1	5	4.3	13	5.7
Moved to urban area	2	1.8	2	1.7	4	1.7
Death	1	0.9	1	0.9	2	0.9
Total	113	100	115	100	228	100

The randomization effect was maintained after the completion of the vaccination schedule (Table 5), eventhough 111 (19,4%) of the 572 participants who received the 3rd dose were lost to follow-up in different periods of time. The main causes of losses were the emigration of the study area and transfer of residence to the urban area (76.6% and 15.3%, respectively). Deaths not related to malaria were responsible for 2.7% and other intercurrent diseases for 1.8%. Three participants (2.7%) were excluded for using impregnated bednets and 1 (0.9%) was vaccinated with the wrong preparation. Fourty six percent of the participants lost to follow-up were vaccinated. The groups were considered homogeneous with respect to the causes of losses (χ^2; p = 0.6).

In this initial approach we concluded that the population selected for the trial was susceptible to infection. Apparently, the participants had different degrees of low acquired immunity, if any at all. The effect of randomization was maintained eventhough the considerable losses to follow-up, which we assume occured at random. Covariates which

Table 5 - Cohort and lost to follow-up baseline characteristics after the completion of the vaccination schedule.

Characteristics	Vaccine					Placebo				
	lost		survivors			lost		survivors		
	n	%	n	%	p*	n	%	n	%	p*
Age										
< 11	6	12	45	19	0.52	10	16	53	24	0.52
11 - 20	20	39	73	31		21	35	75	33	
21 - 50	20	39	98	42		25	42	76	34	
> 50	5	10	20	8		4	7	21	9	
Sex										
Male	28	55	149	63	0.27	36	60	135	60	
Female	23	45	87	37		24	40	90	40	
Time since arrival yrs										
< 1	24	47	87	37	0.16	30	50	81	36	0.10
1 - 2	10	20	44	19		7	12	49	22	
2 - 3	3	6	43	18		7	12	41	18	
> 3	14	27	62	26		16	26	54	24	
Previous malaria episodes										
none7	14	17	7	0.18	9	15	26	12	0.77	
1 - 3	15	29	57	24		14	23	53	23	
several	29	57	162	69		37	62	146	65	
IFA antibodies										
positive	17	33	72	31	0.69	15	25	66	29	0.51
negative	34	67	164	69		45	75	159	71	
Spleen										
palpable	10	20	61	27	0.32	13	22	57	29	0.54
unpalpable	40	80	167	73		45	78	159	71	

*p - value for χ^2 tests for differences between proportions.

indirectly discriminate between susceptibility and exposure of the participants to infection were determined. They will permit to create relevant epidemiologic categories, necessary in future analyses, to adjust estimates of vaccine efficacy or describe effect modifiers.

Analytical approach for estimating vaccine efficacy. The efficacy of a vaccine has been estimated from 1 - RR, where RR is some measure of relative risk. This could be attack rates, incidence rates, or secondary attack rates, among others. Such rates should represent the instantaneous probability of progress to the endpoint of interest (infection, clinical malaria or others) of the vaccinated and unvaccinated given an equal amount of exposure to infection[21]. However, ensuring that comparison populations are subjected to the same exposure of infection is difficult to achieve in the field. It is usual to encounter heterogeneity of exposure to infection in the study population, related to factors such as age, sex, occupation and socio-economic characteristics, among others. Besides, equal exposure to infection is not sufficient to determine a precise and meaningful estimate of the direct effect, since parameter estimates can vary with the amount of exposure. Therefore, the direct effect must be defined with respect to a given amount of exposure to infection. In field studies of most infectious diseases the exposure to infection cannot be controlled by the observer. The term field efficacy applies to situations in the field where the intent is to measure direct effects and attempts are made to control for the amount of exposure. Direct effectiveness measures are those that include some interaction of indirect effects with the efficacy parameter, a situation that is common in field studies of most infectious diseases[5,6,7,8].

Vaccine efficacy trials must also satisfy the exchangeability principle, which implies that the occurrence of the effect would be the same in the untreated as well as in the treated group if they had been treated, and vice versa[6]. On the other hand, this principle can only be achieved if the amount of exposure to infection is known and equal in both treatment groups. In studies which fulfill both conditions, it is possible to determine exposure rates (instantaneous probability of being bitten by infected mosquitos) for vaccinated and non-vaccinated participants. These parameters are necessary for estimating unbiased rates of infection or disease given a certain amount and equal exposure to infection, for both groups, and correspond to the best estimates we can use for estimating the direct biological

effect of the vaccine or vaccine efficacy. Lack of these information leads to the estimation of compound rates that describe the transition of the susceptible stage in vaccinated and unvaccinated to the endpoint of interest. Measures of vaccine efficacy based on compound rates tend to underestimate the efficacy of the intervention[21].

The information gathered in this trial is reported as time to the event of interest (malaria episodes). This will allow us to calculate incidence density rates for both, vaccinated and unvaccinated groups. Baseline transmission, as well as susceptibility to infection of the study participants, will be determined indirectly by defining relevant epidemiologic categories that discriminate between exposure and susceptibility to infection and heterogeneities of both in the study population.

Based on the clinical characteristics of malaria infections in the study population, determined in previous studies, clinical malaria can be easily identified[17]. Therefore, a case of malaria will be define as the presence of *P. falciparum* or *P. vivax* blood-stage parasitemia. A new infection caused by either species will be defined as a positive parasitological examination in a participant observed to be free of parasitemia for at least 30 days since a previous positive slide. With this approach we intend to exclude from the analyses possible treatment failures, relapses or recrudescences. It will also permit to define the time at risk for each participant since both species of plasmodia considered are sensitive, up to a certain extent, to the antimalarials used for treatment for either species.

Estimates of the protective effect of the vaccine will be calculated for the interval between the application of the 2nd and 3rd dose and after the completion of the vaccination schedule. We will consider an induction period of 30 days after the application of each dose. We intend to estimate the overall, as well as the crude and stratum specific estimates of the protective effect of SPf66 for the first and second episodes of *P. falciparum* and *P. vivax* infections separately.

One important issue will be taken into account at this point. Since no radical treatment was administered to the participants before the application of the vaccine or placebo, we can expect that the immune response to the vaccine may be modulated by intercurrent malaria infection. *P. falciparum* and/or *P. vivax* infections detected during the vaccine applications or induction periods, will be considered as covariates in the analyses.

Measures of the protective effect of the vaccine in this trial will be based on compound rates. Attempts will be made in the analyses to control for heterogeneities of exposure and susceptibility to infection in the study population. Therefore, the estimated protective effect that will be obtained will be that of field direct effectiveness. If subjected to correction using previous entomoligical information of the study area, estimates will be closer to the ideal concept of biological efficacy[21].

RESUMO

O presente artigo descreve a população de estudo e o desenho do ensaio de campo de fase III da vacina sintética SPf66 no Brasil. São avaliados os princípios básicos de validade e precisão, essenciais para a estimação adequada da eficácia vacinal. Os resultados da análise exploratória de dados são discutidos assim como, a abordagem analítica para a estimação da eficácia vacinal. Este trabalho fornece o marco conceitual para futuras publicações.

Palavras-chaves: Vacina antimalárica. Ensaio de campo SPf66. Eficácia vacinal.

ETHICAL CONSIDERATIONS

This trial was approved by the ethical committees from the University of Brasília, University of São Paulo and School of Medicine of Uberaba. Written consent was obtained of every participant. Services of the same quality were provided to acceptors and non-acceptors. Malaria control measures were kept unchanged during the vaccine trial.

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