ECONOMIC IMPACT ANALYSIS OF THE USE OF ANTI-DFS70 ANTIBODY TEST IN PATIENTS WITH UNDIFFERENTIATED SYSTEMIC AUTOIMMUNE DISEASE SYMPTOMS

Luca Moroni[1,2], Gabriela Restovic[3], Ricard Cervera[1,4], Gerard Espinosa[1,4], Odette Viñas[3,5], Milagros García[3,5], Laura Sampietro-Colom[6]

[1]Department of Autoimmune Diseases, Hospital Clínic, University of Barcelona, Barcelona, Catalonia, Spain.

[2]Unit of Immunology, Rheumatology, Allergy and Rare Diseases, San Raffaele Scientific Institute, Milan, Italy.

[3]Fundació Clínic per la Recerca Biomèdica, Barcelona, Catalonia, Spain.

[4]Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Catalonia, Spain.

[5]Department of Immunology, Hospital Clínic, University of Barcelona, Barcelona, Catalonia, Spain.

[6]Assessment of Innovations and New Technologies Unit, Hospital Clínic, University of Barcelona, Barcelona, Catalonia, Spain.

Corresponding author:

Ricard Cervera, MD, PhD, FRCP

Department of Autoimmune Diseases

Hospital Clínic

Villarroel, 170

08036-Barcelona, Catalonia, Spain

Tel: +34 93 2275774 / Fax: +34 93 2271707

e-mail: rcervera@clinic.cat

ABSTRACT

OBJECTIVE: In patients with antinuclear antibodies (ANA) and undifferentiated features of systemic autoimmune disease, the coexistence of monospecific anti-dense fine speckled-70 (anti-DFS70) antibodies is associated with a lower risk of progression to overt disease. Therefore, they might help in correctly classifying ANA positive patients and avoiding unnecessary follow-up diagnostic procedures. The aim of this study was to analyze the economic impact of the introduction of the anti-DFS70 antibody test in a hospital setting.

METHODS: A case-control study was performed to detect monospecific anti-DFS70 antibodies in ANA positive subjects with undifferentiated features (cases; n=124) and with a defined systemic autoimmune disease (controls; n=290). Based on current clinical practice, a decision tree was developed to represent the disease course of patients with undifferentiated features in the subsequent three years. A budget impact analysis was performed to estimate the effect of implementing the screening for anti-DFS70 antibodies in the case group on the total costs. A sensitivity analysis was conducted to calculate the impact of the uncertainty of the input parameters on the results.

RESULTS: Among the 124 patients in the case group, 5 (4.0%) tested positive for anti-DFS70 antibodies versus 4/290 (1.4%) in the control group (p=NS). The mean cost per patient under the current clinical practice decreased from 3,274€ to 3,192€ in our scenario. The budget impact reports cost savings of 10,288€.

CONCLUSION: The introduction of anti-DFS70 antibody test would avoid unnecessary follow-up diagnostic procedures and minimize the use of health resources generated by suspicion of a potential systemic autoimmune disease.

KEYWORDS

Anti-DFS70 antibody, undifferentiated connective tissue disease, systemic autoimmune disease, antinuclear antibodies, economic impact.

1. INTRODUCTION

Antinuclear antibodies (ANA) are the hallmark of most systemic autoimmune diseases. Although ANA testing is widely used, its low specificity represents a significant pitfall.[1] Recently, a new ANA specificity, called anti-dense fine speckled (DFS)-70 antibody, has been identified in patients with no or few symptoms of systemic autoimmune disease but with positive ANA by indirect immunofluorescence (IIF).[2] They recognize the 75 kiloDalton lens epithelium derived growth factor (LEDGF), an autoantigen associated with a DFS IIF staining pattern on HEp-2 cells.[1, 3-5]

Anti-DFS70 antibodies are responsible for as much as 12% of positive HEp-2 results in a routine setting,[6] and they have been associated with a wide variety of conditions such as Vogt-Koyanagi-Harada syndrome, atopic dermatitis, and asthma.[2] Currently, they are considered to be more prevalent in patients without systemic autoimmune diseases and healthy individuals,[7, 8] and although their presence cannot exclude the existence of systemic autoimmune disease, the probability is significantly lower.[7] Patients with systemic autoimmune diseases may have anti-DFS70 antibody associated with other immunological markers of autoimmune disease, although the isolated positivity of anti-DFS70 antibody is rare in these patients.[7, 9] Therefore, the presence of isolated anti-DFS70 antibody could be useful to rule out a diagnosis of definite systemic autoimmune disease [10]. In addition, the introduction of anti-DFS70 antibody test as exclusion marker for systemic autoimmune diseases in diagnostic algorithms could be potentially cost-effective.[11] However, in most studies showing epidemiological aspects of the antibody, the information on its economic impact is lacking.[6, 7, 12, 13]

The main purpose of the present study was to determine the prevalence of anti-DFS70 antibodies in a cohort of patients with undifferentiated features of systemic autoimmune diseases with ANA positive but negative antigenic specificity and the economic impact of the implementation of anti-DFS70 antibody test in the management of these patients compared to the current clinical practice.

2. METHODS

2.1. Clinical study design

A clinical retrospective case-control study was carried out at the Hospital Clinic of Barcelona. The clinical study was designed to assess the prevalence and clinical associations of anti-DFS70 antibodies in ANA positive patients without systemic autoimmune diseases (cases) and with definite systemic autoimmune diseases (controls). Patients in both groups were chosen consecutively among those attending the outpatient clinic at the Department of Autoimmune Diseases of the Hospital Clinic of Barcelona from January 1st to December 31st, 2016.

2.1.1. Patients: Cases were defined as patients with positive ANA (HEp-2) at significant titer (≥ 1:80), negative for antigenic specificities including anti-dsDNA, anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-RNP, anti-Scl70, anti-centromere, anti-RNA-polymerase III, and anti-PM/SCl antibody, and with a clinical suspicion of systemic autoimmune disease but without a definite diagnosis over 6 months from the beginning of the symptoms. This case definition greatly overlaps the most important proposed classification criteria for undifferentiated connective tissue disease (UCTD).[14-17]

Four groups of patients were considered as control group and included those fulfilling the currently proposed classification criteria of systemic lupus erythematosus

(SLE)[18], Sjögren's syndrome (SS)[19], systemic sclerosis (SSc)[20], and antiphospholipid syndrome (APS).[21]

The study was approved by the local institutional review board (approval number: HCB/2016/0790) that waived the requirement for individual informed consent.

2.1.2. Variables: Clinical variables for the cases included symptoms or signs suggestive of systemic autoimmune diseases such as Raynaud's phenomenon, arthralgia, arthritis, fatigue, skin lesions, oral and genital ulcers, photosensitivity, fever, sicca syndrome, serositis, leucopenia, thrombocytopenia, livedo reticularis, thrombosis, and obstetric morbidity including early miscarriage (≤ 10 weeks of pregnancy) and late abortions or fetal loss (> 10 weeks of pregnancy). Considering controls, the main clinical manifestations of the previously defined systemic autoimmune diseases were also collected. For cases and controls, comorbidities, associated organ-specific autoimmune diseases, and malignancies were registered. Follow-up was defined as the time (in months) from the beginning of the symptoms to the last visit. Immunological profiles, number of visits and diagnostic imaging techniques in cases and controls, following the current routine care protocols established for each disease, were recorded to describe the health resources used by each group.

2.1.3. Chemiluminescence anti-DFS70 antibody assays: In all cases and controls, anti-DFS70 antibody determination by chemiluminescence (QUANTA Flash Inova Diagnostics, CA, USA) was performed. The QUANTA Flash DFS70 assay is a novel chemiluminescence immuno-assay that uses recombinant DFS70 expressed in *E. coli* coated onto paramagnetic beads and is designed for the BIO-FLASH instrument (Biokit S.A., Barcelona, Spain)[7]. The relative light units (RLUs) are proportional to the amount of isoluminol conjugate that is bound to the anti-human IgG, which in turn is

proportional to the amount of anti-DFS70 antibodies bound to the antigen on the beads. Using a standard curve, all RLU values are converted into calculated units (CU). Samples with antibody titers above the analytical measuring range (AMR, 3.2-450.8 calculated units, CU, cutoff = 20 CUs) were prediluted 1:20 and retested to determine the exact anti-DFS70 antibody concentration.

2.1.4. Statistical analysis: Descriptive statistics were used to summarize all demographic and laboratory variables of both groups. Comparison of frequencies among groups for categorical parameters was performed by $\chi 2$ and Fisher exact tests. Nonparametric Kruskal-Wallis test was employed to compare multiple median values of continuous non-normal variables among groups. Spearman's rank model was used for correlation analysis. ROC curves were built to analyze diagnostic performance of biomarkers and scores. P values < 0.05 were considered significant.

2.2. Economic analysis

Based on the results and the information collected in the clinical retrospective case-control study (i.e., the prevalence of anti-DFS70 antibodies in ANA positive patients without systemic autoimmune diseases, health resources used per patients and its unit costs), two types of economic analyses were carried out to estimate the impact of introducing the anti-DFS70 antibody test as diagnostic tool in the clinical practice.

The **first analysis was a mean cost per patient analysis**. A decision tree was developed [22] to represent the disease course in the next three years of patients with symptoms of undifferentiated systemic autoimmune diseases from the moment they have been referred to the hospital to be diagnosed, the branches of the initial decision node represent the strategies to be compared, in this analysis we are comparing the current

clinical management at the hospital ("current clinical practice scenario without anti-DFS70") with the expected clinical management at the hospital after including the anti-DFS70 antibody test ("expected scenario including anti DFS70) (**Figure 1**).

Literature data show clearly that patients with an undifferentiated onset will have three potential clinical courses: evolving to resolution of symptoms after 3 years follow-up, remaining with unspecific symptoms after 3 years follow-up, or developing a defined systemic autoimmune disease after 3 years follow-up (23). When the anti-DFS70 antibody test is included in the pathway of care to identify the presence of the systemic autoimmune disease in all patients with signs and symptoms of these diseases, a new potential health state is included in the disease course. This new health state - named "anti-DFS70 positive with low possibility to develop a systemic autoimmune disease" - appears when patients tested positive for anti-DFS70 antibody test.

For all the health states (excluding the abovementioned new health state), economic variables, considering the type and frequency of resources used for patient, were obtained from hospital records within the case-control study and validated by clinical experts (RC, GE). Economic variables included were: laboratory tests, visits (first and follow-up), diagnostic imaging, and costs related with the derivations to other physicians. In this way, mean costs per health state were calculated. For the new health state "positive anti-DFS70 antibody linked to low possibility to develop a systemic autoimmune disease", the type of resources to be used by these patients were obtained from the clinical experts opinion, and they include: a first visit to specialist, one follow-up visit, one test including blood cell count, hepatic and renal

function tests, urinalysis, ANA HEp-2, anti-dsDNA/*Crithidia luciliae*, anti-centromere, anti-Scl70, anti-Ro/SSA, anti-LA/SSB, anti-Sm/RNP antibodies, and C3, C4 and CH50 levels. Moreover, clinical experts agree that, under this new health state, the patient will be discharged and not be followed systematically during the next three years. The transition probabilities for each health states (probability of moving from one state to another) to populate the decision tree, were mainly obtained from the results of the clinical case-control study. When no data was available, information from the literature along with clinical expert judgment was used.

The **second analysis was a cohort BIA**, which is based on estimating the difference in the total costs per a cohort of patients using current clinical practice scenario or after the introduction of the anti-DFS70 antibody test as diagnostic tool (expected scenario). The clinical parameter used here was the prevalence of anti-DFS70 antibody found in the case group of the clinical study. The total costs for current clinical practice scenario were computed by, first, considering the three health states described above, then, multiplying by the average per-patient costs for each health state and, finally, summing across the results from each branch of the decision tree model. The total costs for the expected scenario (introducing anti-DFS70 antibody test) were estimated in the same way adding the cost per patient of the already described new health state ("anti-DFS70 positive linked to low possibility to develop a systemic autoimmune disease"). The difference between both scenarios allowed the estimation of the hospital budget impact.

Both analyses were performed from the perspective of the Hospital Clinic of Barcelona (considering only direct costs) and developed using Microsoft Excel. Costs not directly related to the diagnosis of the systemic autoimmune disease were not considered.

Unit costs associated to the resources used were obtained from hospital database and are expressed in 2018 Euros (€). No discount rate was applied. The price of anti-DFS70 antibody test for the Hospital Clinic Barcelona was used.

One-way deterministic sensitivity analysis was conducted to analyse the impact of the uncertainty of economic and clinical parameters on the final results. Sensitivity analysis is an important part of the evaluation process and gives valuable information to decision-makers about the robustness of the findings of an economic evaluation, as well as the potential value of collecting more information before making a final decision.[24]

The main input parameters used in the budget impact analysis (% of patients with positive anti-DFS70 antibody, % of patients evolving to resolution of symptoms after 3 years follow-up, % of patients remaining with unspecific symptoms after 3 years follow-up, % of patients developing a systemic autoimmune disease after 3 years follow-up, and unit cost of the anti-DFS70 antibody test) were varied in a range of uncertainty. This was designed as a tornado graphic representation, which highlights the parameters that may mostly affect the final results if the figures used in our analysis are different in other settings. The range of uncertainty (variation interval) for each parameter was determined based on a range of variability equal to ± 20% of the central value, which represents a reasonably wide range.

3. RESULTS

3.1. Clinical results from case-control study

Overall, 414 patients (124 cases and 290 controls) were included in the study. Four groups of patients were considered as control group including 91 patients with SLE, 82

with SS, 58 with SSc, and 59 with APS. Demographic characteristics of cases and controls are described in **Supplementary File 1**. As expected, case and control groups are female-predominant. The main clinical features of the patients in case and control groups are summarized in **Table 1A**. The most common "undifferentiated" symptom was arthralgia (40.3%), followed by Raynaud phenomenon (32.2%), and sicca syndrome (31.5%). Distribution of ANA titers, ANA patterns, and ENA specificities by group are summarized in **Table 1B.**

3.2. Prevalence of anti-DFS70 antibody

Considering the manufacturer cutoff value, 5 (4.0%) patients among the 124 in the case group tested positive for anti-DFS70 antibody versus 4/290 (1.4%) in the control group (one for each disease, respectively) (p=NS). All 5 anti-DFS70 positive subjects in the case group showed homogeneous or homogeneous-speckled patterns (**Figure 2**). None of the 4 patients who tested positive for anti-DFS70 antibody in the control group resulted to be "mono-specific" and displayed either at least two extractable nuclear antigen (ENA) specificities other than anti-DFS70 or anti-thyroperoxidase antibody (**Supplementary File 2**). No statistically significant difference in clinical profile was found in cases grouped by anti-DFS70 antibody status, neither in anti-DFS70 titer between positive cases and controls (163.7 CU versus 99.8 CU, respectively). Individual values of Quanta Flash DFS70 assay are reported in **Table 1C** showing that none of the anti-DFS70 positive cases were displaying highest titers (1:640 or above).

3.3. Mean cost per patient analysis

The transition probabilities for each health states depending on the results of anti-DFS70 antibody (positive or negative) are shown in **Table 2**. The mean costs per patient under the current clinical practice scenario and under the expected scenario including anti-DFS70 antibody as diagnostic tool (i.e. expected scenario), are shown in **Table 3**. In the base case, the mean costs per diagnosed patient were 3,274€ under current clinical practice and 3,192€ when anti-DFS70 antibody test was incorporated as diagnostic tool. Savings associated with the anti-DFS70 antibody test were fully explained by the lower health resources used by patients that tested positive; these patients are discharged after the testing, saving health resources by not requiring additional laboratory tests, diagnostic imaging test and/or visits in the following three years.

3.4. Cohort budget impact analysis

The results of the 3 year-follow-up of patients in each scenario are shown in **Table 4**. For the studied population, namely 124 patients with signs and symptoms of undifferentiated systemic autoimmune disease, under the current clinical practice, the total cost would be 135,323€ per year for the next 3 years. The main cost corresponds to the laboratory test used in the diagnosis and monitoring of patients with positive ANA (HEp-2) at significant titer (1:80) without specific antibodies and without associated systemic autoimmune disease. For the expected scenario, the total costs would be 136,108€ in the first year and 129,866€ in the following two years. Therefore, the introduction of the anti-DFS70 antibodies as diagnostic tool will have a cost reduction of 10,128€ during the 3 years management for the total patients analyzed in the study.

The deterministic one-way sensitivity analysis (**Figure 3**) showed that the results were robust with no single variable modifying the finding that the anti-DFS70 antibody test introduction as diagnostic tool for patients with undifferentiated systemic autoimmune disease was cost saving compared with the current clinical practice. It is important to notice that the variable that most impact can have in the differences of total costs that have been found between the two scenarios is the prevalence of patients with anti-DFS70 antibody; the higher the prevalence the greater the cost reduction in the management of patients with undifferentiated systemic autoimmune disease.

4. DISCUSSION

Our study demonstrated that the introduction of the anti-DFS70 antibody test in the initial work-up of patients with suspected systemic autoimmune disease would avoid unnecessary follow-up visits and minimize the use of health resources generated by suspicion of a potential systemic autoimmune disease.

Anti-DFS70 antibody displays its highest predictive value when its finding is isolated.[8, 12, 25] Mariz et al,[8] reported that none of 40 healthy individuals with isolated anti-DFS70 reactivity developed a systemic autoimmune disease within an average 4-year follow-up. In our study, a 3-year follow-up appeared to be sufficient to discard evolution to an overt systemic autoimmune disease according to previous studies on undifferentiated connective tissue disease natural history [26-31]. Fitch-Rogalsky et al.[25] reported that anti-DFS70 antibody positive subjects have a positive likelihood ratio (LR+) of non-systemic autoimmune rheumatic disease of 5.4 and when considered the LR+ increases to 10.9 when found isolated. These data were recently

confirmed by Shovman et al.[12], demonstrating that prevalence of monospecific anti-DFS70 antibodies was significantly higher in healthy subjects than in patients with ANA-related autoimmune diseases (10.9% vs. 1.9%, p=0.02). Our results are consistent with the literature since none of the control subjects was found to be anti-DFS70 monospecific.

Only homogeneous, speckled, or homogeneous-speckled ANA patterns (AC-2 International Consensus on Autoantibody Patterns (ICAP) patterns) are considered to be consistent with anti-DFS70 antibody. These data easily lead to the conclusion that narrowing the population to which the test is applied increases its potential value. In fact, the most appropriate role of anti-DFS70 antibody screening would be ruling out autoimmune disorders in subjects with low pre-test probability of a systemic autoimmune disease, ANA positive with consistent pattern, and negative ENA or anti-thyroperoxidase antibodies.

Pre-test probability of a systemic autoimmune disease strongly depends on clinical judgment, that remains the cornerstone of the diagnosis, but it is also influenced by the clinical setting. [32,33] Within populations where the pre-test probability of a systemic autoimmune disease is generally low, such as in primary care, the added value of a positive anti-DFS70 antibody test would be worthier to exclude a systemic autoimmune disease, if compared to secondary and tertiary care. Our study was performed in a tertiary level center and, as expected, overall prevalence of anti-DFS70 antibody is low in all groups. These differences among clinical settings have been recently reproduced in a Belgian study that compared anti-DFS70 antibody prevalence among sera obtained from primary, secondary, and tertiary care laboratories.[34]

With respect to patients with a defined systemic autoimmune disease, the proportion of anti-DFS70 antibody positivity is similar to those found in SLE (0-6%)[9, 13, 35-37] and SSc (0.6-2.5%)[9, 13, 36] but significantly lower for SS (1.2% vs. 11.3%)[9]. Our choice to introduce APS as a study group can be puzzling, since APS is not generally ANA-related. However, previous reports of potential association between anti-DFS70 antibody and APS are of interest. [38] In our cohort, no difference in neither obstetric morbidity nor thrombosis incidences have been outlined in the case group. Considering our data together with the qualified literature, the association of anti-DFS70 antibody with APS manifestations appears unfounded.

Regarding to the economic perspective, Gundín S et al.[11] compared a conventional algorithm used at a hospital setting in Spain for the diagnosis of patients with suspicion of ANA-associated autoimmune diseases versus a new algorithm including anti-DFS70 antibody detection. They considered two types of costs: the laboratory ANA and follow-up testing, and the resulting clinic visits. The authors found that the use of the new algorithm resulted in a cost saving of 60,869€. The incidence of positive anti-DFS70 antibodies was reported in 12.7% for the total population under study. This finding would be in line with our sensitivity analysis in which we demonstrated that at a higher incidence of positive anti-DFS70 antibody tests the economic impact would be greater. In fact, prevalence of anti-DFS70 positivity is the key variable of the model, suggesting even greater savings if implemented in settings like primary care, where it is likely to be higher (Tornado diagram, **Figure 3**). This is undoubtedly the real strength of our study.

The main limitation of this study is the observational design that did not allow having a real but only “simulated” timeline. Furthermore, additional groups of patients with

other systemic autoimmune diseases (i.e., rheumatoid arthritis, autoimmune inflammatory myopathies, ANCA-associated vasculitis…) should be considered as control group. Future prospective studies would clarify whether our data are confirmed.

In conclusion, isolated anti-DFS70 antibody test represents a potentially biomarker that can be used clinically to discriminate systemic autoimmune diseases from other conditions in ANA positive individuals, better if used in a proper referral context, and would help avoiding unnecessary follow-up visits and costs. For that reason, algorithms containing anti-DFS70 antibody tests will allow to reduce unnecessary tests and follow-up visits generated by the suspicion of an unlikely autoimmune disease in the upcoming years.

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Competing interest None.

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Transparency statement The authors affirm that the manuscript is honest, accurate and in accordance with the pre-specified protocol. No important aspects of the study have been omitted in the current manuscript.

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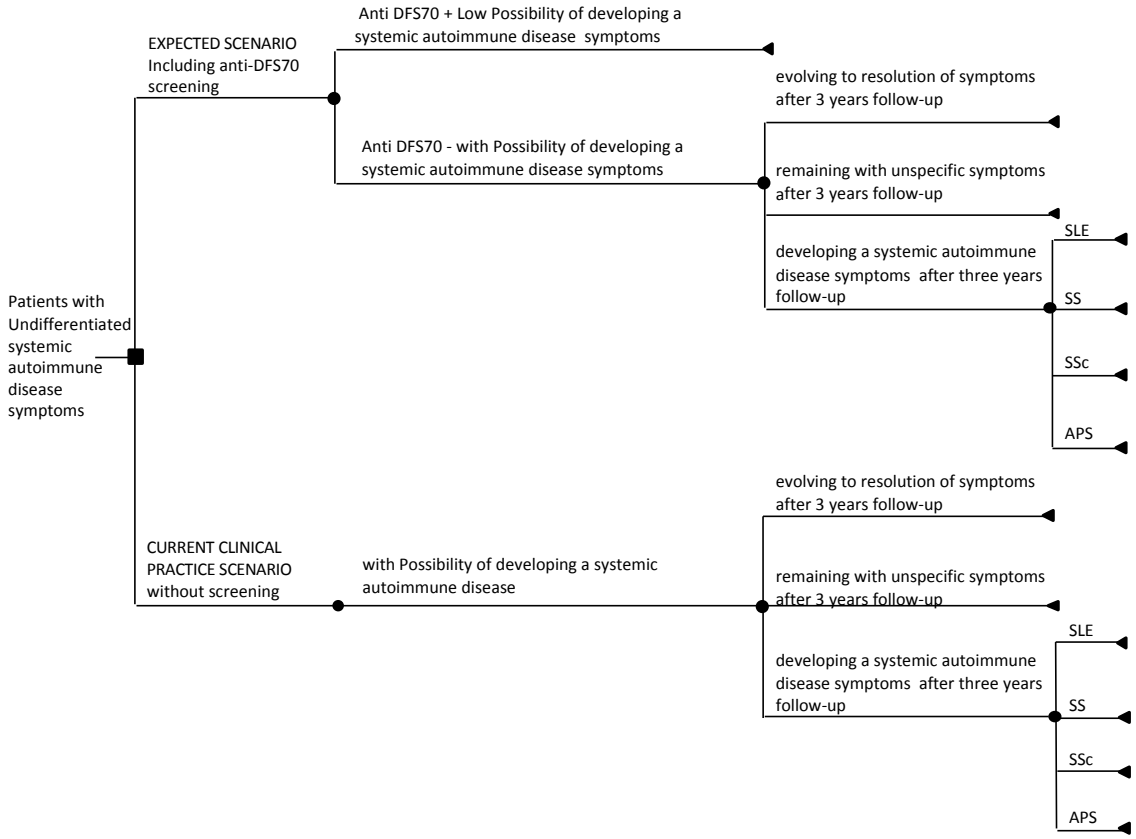
Figure 1. Decision tree showing the disease course (i.e. health states) in patients with undifferentiated systemic autoimmune disease symptoms under current clinical practice and when the anti-DFS70 test is used.



Legend: The decision node (□) shows the comparative analysis of alternative courses of care in front of a patient with undifferentiated systemic autoimmune disease symptoms. The two courses of care alternatives are to include the anti DFS70 antibody test to detect patients with a low possibility to develop a systemic autoimmune disease or the current clinical practice (with no anti DFS70). For each alternative, a series of chance nodes (•) are possible. The outcomes (i.e. costs and number of patients) are estimated at the end of each pathway (∇)

Abbreviations: APS: antiphospholipid syndrome); SLE: systemic lupus erythematosus; SS: Sjögren syndrome; SSc: systemic sclerosis.

Figure 2. Immunofluorescence pattern on HEp-2 substrate (INOVA Diagnostics). Left, case serum with a DFS70 value of 248.7 and ANA titer 1:80. Right, disease control serum from a patient with Sjögren's syndrome, DFS70 value of 237.2 and ANA titer 1:320.

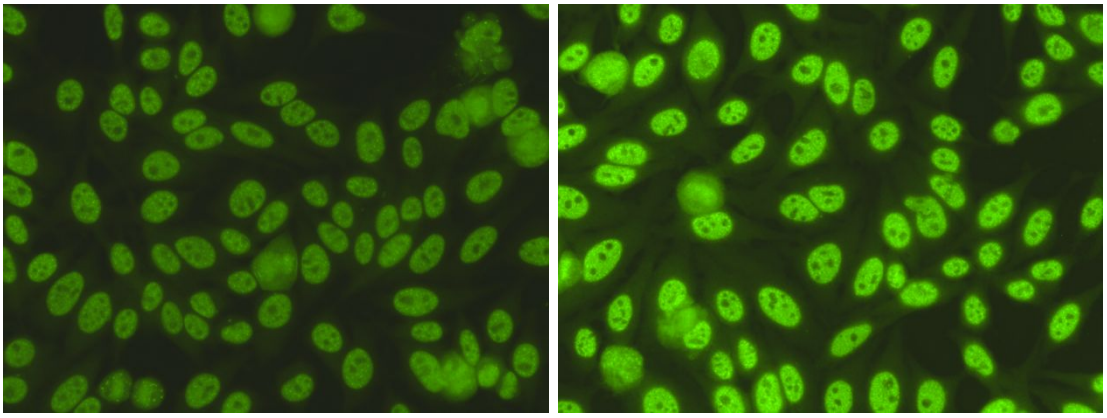


Figure 3. Budget Impact sensitivity analysis with a tornado diagram – 10,128€ base case.

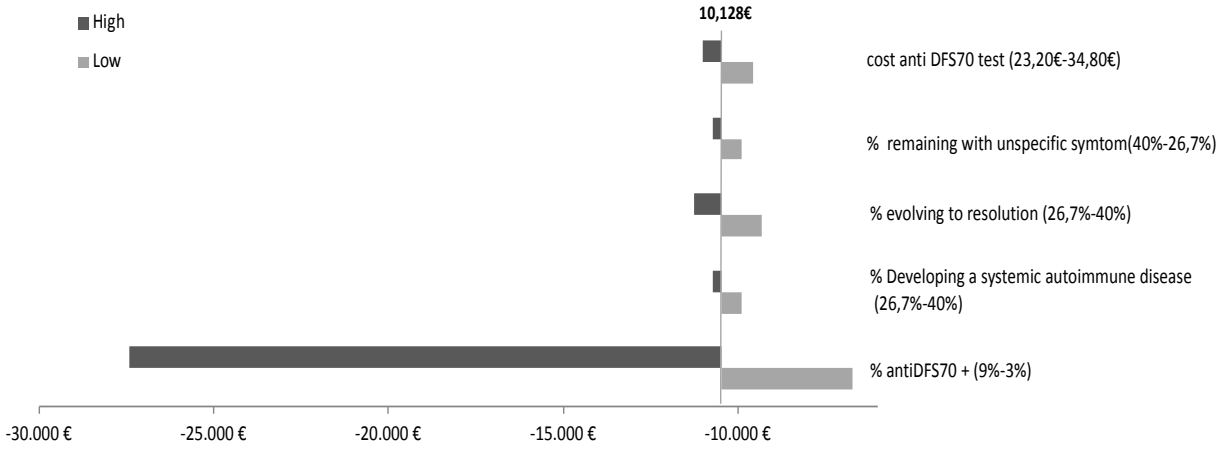


Table 1A. Clinical manifestations of the studied population

CASES (N=124)	**N (%)**
Arthralgia	50 (40.3)
Raynaud phenomenon	40 (32.2)
Sicca syndrome	39 (31.5)
Fatigue	28 (22.6)
Leucopenia	26 (21.0)
Obstetric morbidity	26 (21.0)
Arthritis	19 (15.3)
Skin lesions	18 (14.5)
Aphthous ulcers	17 (13.7)
Photosensitivity	14 (11.3)
Fever	14 (11.3)
Thrombocytopenia	9 (7.3)
Serositis	6 (4.8)
Thrombosis	6 (4.8)
Livedo reticularis	2 (1.6)
SYSTEMIC LUPUS ERYTHEMATOSUS (N=91)	
Articular involvement	75 (82.4)
Cutaneous involvement	68 (74.7)
Hematological involvement	46 (50.5)
Renal involvement	44 (48.4)
Serositis	22 (24.2)
Raynaud phenomenon	11 (12.1)
Fever	9 (9.9)
Neuropsychiatric lupus	6 (6.6)
SJÖGREN'S SYNDROME (N=82)	
Glandular involvement	64 (78.0)
Extraglandular involvement	18 (22.0)
SYSTEMIC SCLEROSIS (N=58)	
Limited systemic sclerosis	34 (58.6)
Sine scleroderma	14 (24.1)
Diffuse systemic sclerosis	8 (13.8)
Pre-scleroderma	2 (3.4)
Raynaud phenomenon	56 (96.6)
Gastrointestinal involvement	41 (70.7)
Interstitial lung disease	19 (32.8)
Articular involvement	15 (25.9)
Pulmonary hypertension	10 (17.2)
ANTIPHOSPHOLIPID SYNDROME (N=59)	
Thrombotic	45 (76.3)
Obstetric	26 (44.1)

AUTOANTIBODIES		(%) Cases (n=124)	SLE (n=91)	SS (n=82)	SSc (n=58)	APS (n=59)
ANA titer	1:40	0,0	1,1	9,8	5,2	5,1
	1:80	29,0	3,3	9,8	8,6	15,3
	1:160	36,3	19,8	17,1	27,6	27,1
	1:320	20,2	17,6	24,4	22,4	22,0
	≥1:640	14,5	58,2	39,0	36,2	30,5
ANA pattern*	homogeneous	72,5	91,3	68,2	58,4	86,4
	speckled	58,0	55,0	70,7	36,1	74,6
	centromeric	0,0	0,0	1,2	53,4	0,0
	nucleolar	22,4	13,2	31,7	31,0	20,5
	few nuclear dots	2,4	0,0	0,0	0,0	0,0
	p80-coilin	1,6	0,0	0,0	0,0	0,0
Rheumatoid factor		3,2	15,4	29,3	12,1	5,1
Anti-dsDNA		0,0	53,8	6,1	10,3	18,6
Anti-Ro52		0,0	36,3	26,7	10,3	0,0
Anti-Ro60		0,0	9,9	68,3	8,6	5,1
Anti-La		0,0	19,8	46,3	1,7	0,0
Anti-Sm		0,0	23,1	4,9	0,0	5,1
Anti-RNP		0,0	1,1	11,0	5,2	10,2
Anti-Scl70		0,0	0,0	2,4	17,2	0,0
Anti-centromere		0,0	0,0	3,7	53,4	1,7
Anti-RNA Pol III		0,0	18,7	0,0	10,3	0,0
Anti-PM/Scl		0,0	0,0	0,0	5,2	0,0
LAC		0,0	27,5	4,9	6,9	79,7
aCL IgG		0,0	8,8	2,4	3,4	76,3
aCL IgM		0,0	4,4	3,7	7,0	40,7
Anti-beta2GPI IgG		0,0	4,4	2,4	3,4	33,9
Anti-beta2GPI IgM		0,0	4,4	3,7	5,2	28,8
Anti-TPO (≥ 35)		13,4	12,5	12,5	17,4	30,0
Anti-TG (≥ 60)		17,2	26,7	29,2	22,7	20,0

Table 1B. Autoantibody distribution in the study population by disease group. *Mixed patterns are included and counted twice.

Table 1C. Individual values of Quanta Flash anti-DFS70 assay with respective titer of ANA IIF

Anti-Hep-2 indirect immunofluorescence (IIF)				Quanta Flash	Group
ANA titer	**ANA pattern**	**Cytoplasmic titer**	**Cytoplasmic pattern**	**Anti-DFS70 value (CU)**	
1:80	homogeneous-speckled*	negative		25,8	Case
1:320	homogeneous-speckled*	negative		198,8	Case
1:80	homogeneous-speckled*	negative		248,7	Case
1:160	homogeneous-speckled*	negative		101,5	Case
1:320	homogeneous-speckled*	negative		243,6	Case
1:640	homogeneous and speckled	negative		56,4	SLE
1:640	speckled (and homogeneous 1:80)	negative		237,2	SS
1:640	centromeric (and homogeneous 1:40)	1:160	granular**	79,9	SSc
1:160	nucleolar	negative		23,6	APS

* the observed pattern is compatible with the description of the DSF described pattern and the suspicion of the presence of anti-DSF70 antibodies
** this sample is positive by Dot-Blot for AMA-M2

Table 2. Health states probabilities transitions used in the Decision Tree Model and BIA

Expected scenario including anti-DFS70 screening	**Probability**	**Source**
Anti-DFS70 + Low Possibility of developing a systemic autoimmune disease	0.04	Case-control study
Anti-DFS70 - with Possibility of developing a systemic autoimmune disease	0.96	Case-control study
Evolving to resolution of symptoms after 3 years follow-up	0.33	Clinical experts, Bodalay et al[32]; Danieli et al.[33]
Remaining with unspecific symptoms after 3 years follow-up	0.33	
Developing a systemic autoimmune disease after three years follow-up	0.33	
SLE	0.30	Case-control study
SS	0.27	
SSc	0.22	
APS	0.22	
Current clinical practice scenario (without anti-DSF70 screening)	**Probability**	**Source**
Anti-DFS70 - with Possibility of developing a systemic autoimmune disease	1.00	Current practice
Evolving to resolution of symptoms after 3 years follow-up	0.33	Clinical experts and Bodalay et al.[32]; Danieli et al.[33]
Remaining with unspecific symptoms after 3 years follow-up	0.33	
Developing a systemic autoimmune disease after three years follow-up	0.33	
SLE	0.30	Case-control study
SS	0.27	
SSc	0.22	
APS	0.22	

Abbreviations: APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; SS: Sjögren syndrome; SSc: systemic sclerosis

Table 3. Mean cost per patient per clinical scenarios (current and expected) for three years.

Potential disease course	Anti DFS 70 Test	Other Lab test	Visits	Diagnostic Imaging	Derivations	Total Cost per Health State
Anti-DFS70 + Low Probability of developing a systemic autoimmune disease	29 €	255 €	274 €	0 €	0 €	558 €
Evolving to resolution of symptoms after 3 years follow-up	29 €	961 €	822 €	0 €	0 €	1,812 €
Remaining with unspecific symptoms after 3 years follow-up	29 €	2,359 €	1,233 €	636 €	411 €	4,668 €
Developing a systemic autoimmune disease after three years follow-up (SLE/SS/SSc/APS)	29 €	2,156 €	771 €	422 €	51 €	3,429 €
Mean costs expected scenario – including anti-DFS70 screening						**3,192€**
Evolving to resolution of symptoms after 3 years follow-up	0 €	961 €	822 €	0 €	0 €	1,783 €
Remaining with unspecific symptoms after 3 years follow-up	0 €	2,359 €	1,233 €	636 €	411 €	4,639 €
Developing a systemic autoimmune disease after three years follow-up (SLE/SS/SSc/APS)	0 €	2,156 €	771 €	422 €	51 €	3,400 €
Current clinical practice scenario - without screening anti-DSF70 test						**3,274€**

Abbreviations: APS: antiphospholipid syndrome; SLE: systemic lupus erythematosus; SS: Sjögren syndrome; SSc: systemic sclerosis

Table 4. Budget impact analysis

ELIGIBLE POPULATION	Year 1	Year 2	Year 3
CURRENT SCENARIO - CLINICAL PRACTICE			
	Year 1 (n=124)	**Year 1 (n=124)**	**Year 1 (n=124)**
Patient with low probability of developing systemic autoimmune disease	**0 €**	**0 €**	**0 €**
Cost of other lab test	0 €	0 €	0 €
Cost of visits	0 €	0 €	0 €
Cost of diagnostic imaging	0 €	0 €	0 €
Cost of derivations	0 €	0 €	0 €
	Year 1 (n=124)	**Year 2 (n=124)**	**Year 3 (n=124)**
Patient with probability of developing systemic autoimmune disease	**135,323 €**	**135,323 €**	**135,323 €**
Cost of other lab test	75,441 €	75,441 €	75,441 €
Cost of visits	38,931 €	38,931 €	38,931 €
Cost of diagnostic imaging	14,580 €	14,580 €	14,580 €
Cost of derivations	6,371 €	6,371 €	6,371 €
Total costs CURRENT SCENARIO - CLINICAL PRACTICE	**135,323 €**	**135,323 €**	**135,323 €**
EXPECTED SCENARIO - INCLUDING ANTI-DFS70 SCREENING TEST			
	Year 1 (n=5)	**Year 2 (n=0)**	**Year 3 (n=0)**
Patient with low probability of developing systemic autoimmune disease	**2,791 €**	**0 €**	**0 €**
Cost Anti-DFS70 diagnostic test	145 €	0 €	0 €
Cost of other lab test	1,276 €	0 €	0 €
Cost of visits	1,370 €	0 €	0 €
Cost of diagnostic imaging	0 €	0 €	0 €
Cost of derivations	0 €	0 €	0 €
	Year 1 (n=119)	**Year 2 (n=119)**	**Year 3 (n=119)**
Patient with probability of developing systemic autoimmune disease	**133,317 €**	**129,866 €**	**129,866 €**
Cost Anti-DFS70 diagnostic test	3,451 €	0 €	0 €
Cost of other lab test	72,399 €	72,399 €	72,399 €
Cost of visits	37,361 €	37,361 €	37,361 €
Cost of diagnostic imaging	13,992 €	13,992 €	13,992 €
Cost of derivations	6,114 €	6,114 €	6,114 €
Total costs EXPECTED SCENARIO INCLUDING ANTI-DFS70 SCREENING TEST	**136,108 €**	**129,866 €**	**129,866 €**
BUDGET IMPACT	**785 €**	**-5,457 €**	**-5,457 €**