Preliminary results of the project A.I.D.A. (Auto Immunity: Diagnosis Assisted by computer)

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Abstract: - In this paper, are presented the preliminary results of the A.I.D.A. (Auto Immunity: Diagnosis Assisted by computer) project which is developed in the frame of the cross-border cooperation Italy-Tunisia. According to the main objectives of this project, a database of interpreted Indirect ImmunoFluorescence (IIF) images on HEp 2 cells is being collected thanks to the contribution of Italian and Tunisian experts involved in routine diagnosis of autoimmune diseases. Through exchanging images and double reporting; a Gold Standard database, containing around 1000 double reported IIF images with different patterns including negative tests, has been settled. This Gold Standard database has been used for optimization of a computing solution (CAD-Computer Aided Detection) and for assessment of its added value in order to be used along with an immunologist as a second reader in detection of auto antibodies for autoimmune disease diagnosis. From the preliminary results obtained, the CAD appeared more powerful than junior immunologists used as second readers and may significantly improve their efficacy.

Key-Words: - Autoimmunity, ImmunoFluorescence, Database, Gold Standard, CAD, Patterns, Accuracy.

1 Introduction

Autoimmune diseases are due to reaction of immune system to self antigens, occurring through loss of tolerance. The targeted antigens might be common to all kinds of cells or organ specific, and their recognition by humoral or cellular immune effectors could lead to diversified symptoms, depending on pathology. Presence of auto-antibodies in patient sera has a diagnosis value and establishment of their titer and specificity helps to confirm the auto-immune disease and its follow-up. Screening of non organ specific auto-antibodies (particularly antinuclear antibodies) in sera is performed by immunologists using a routine technique based on Indirect ImmunoFluorescence on HEp2 cells (IIF). The binding of auto-antibodies on HEp2 cells is revealed by fluorescent antibodies against human immunoglobulins. The fluorescence pattern observed under microscope (Homogeneous, Fine Speckled, Coarse Speckled, Nucleolar, Centromere, Nuclear Dots, etc.) is characteristic of

ISBN: 978-960-474-401-5

the nature of the self antigen and of its location in the cell. The difficulty of IIF diagnosis technique is related to the distinction of very close fluorescence patterns (such as Fine Speckled, and Coarse Speckled patterns) and to the subjectivity of the observer. For that reason, two senior immunologists (double lecture) with strong experience in fluorescent image interpretation are needed. However this condition is not respected in all immunology laboratories involved in diagnosis.
The need of the scientific community for a large database of IIF images reported out by medical experts is increasing. Its use could be related to various purposes: training of young immunologists, epidemiological studies, diagnosis, etc. Storing, processing and sharing such data require necessarily computer techniques [1]. Moreover, other computing programs are needed in order to avoid difficulties of IIF images interpretation. As it is done in other medical areas (eg Radiology), that face the same kind of problems, the second reader could be replaced by a computing solution (CAD-Computer Aided Detection) [2,3]
In this paper is presented the A.I.D.A. (Auto Immunity: Diagnosis Assisted by computer) project and its preliminary results. Financed by cross-border cooperation Italy-Tunisia, involving four teams in Sicily and four teams in Tunis, this project has two main objectives: *setting a big database of interpreted IIF images thanks to the contribution of Italian and Tunisian hospitals and *evaluation of the added value of the CAD, to be used along with an immunologist as a second reader in detection of auto-antibodies.

2 Materials and methods

2.1 The AIDA project and Partners involved

The project A.I.D.A (Autoimmunity: diagnosis assisted by computer) is financed by cross-border cooperation Italy-Tunisia, involving four teams in Sicily and four teams in Tunis as presented below. Other associated partners have been involved in the project, the duration of which is 30 months starting from November 2012.
The Partners of the project are:

- University of Palermo - Department of Physics and Chemistry, Palermo, Italy (Coordinator)
- Department of Health of the Sicilian Region, Palermo, Italy.
- Regional Province of Agrigento, Italy.
- ASP-TP, U.O.C. of Clinic Pathology P.O. of Trapani, Trapani, Italy.
- Faculty of Sciences of Tunis University of Tunis El Manar, Tunis, Tunisia
- Pasteur Institute of Tunis, Tunisia.
- Charles Nicolle Hospital, Tunis, Tunisia.
- Public Health Department, Tunis, Tunisia.

Other partners associated or collaborators to the project are the following:

- Regional Department of Production Activities, Sicily- Italy
- TECHNOPARK of Sidi Thabet – Tunisia
- Hospital of Ariana, Tunis, Tunisia.
- Hospital Buccheri La Ferla, Palermo, Italy
- Hospital Civico, Palermo, Italy
- ASP-AG, U.O.C. of Clinic Pathology P.O. of Sciacca, Agrigento, Italy.

2.2 IIF technique and protocol

IIF technique has been performed on HEp2 cells, using patients sera addressed to immunology laboratories for detection of anti-nuclear auto-antibodies. After incubation of the 1/80 serum dilution, bound antibodies are revealed by fluorescent antibodies to human immunoglobulin. The negativity or positivity of the serum is established along with the fluorescence pattern (Homogeneous, Fine Speckled, Coarse Speckled, Nucleolar, Centromere, Nuclear Dots, etc.) which is indicative of auto-antibody specificity. At least one image is taken for negative sera, and three images are taken for positive sera.

2.3 Database and readers

Using homogenized approach, three Tunisian and four Sicilian immunology services contributed to obtain images of IIF test on HEp2 cells. These images correspond to the routine IIF technique performed in the different hospitals for auto-immune diseases diagnosis and were, hence interpreted by senior immunologists. A total of 5762 sera of patients addressed for diagnosed of autoimmune disease were involved in this study as indicated in the following table 1.

Table 1: Number of sera and images

Number of patients	Results of IIF test	Number of images taken	Total images
5762	4316 Positive	12947	14393
	1446 negative	1446	

ISBN: 978-960-474-401-5

Each image with the report was stored in a common database created in the frame of A.I.D.A. project. The Database cumulative contents of two years activity are given month by month in figure 1 and reached 14393 images stored in the database. This number is very high as compared to public IIF images databases available in the world [4].

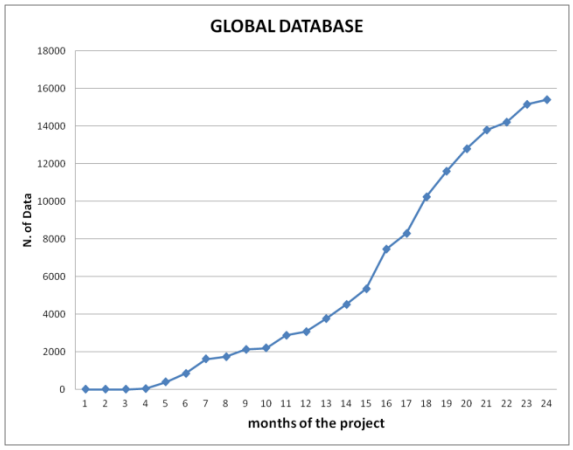

Fig. 1: Cumulative number of IIF images introduced along with reporting in A.I.D.A. database.

Among the stored images, 1446 (around 10%) were considered as negative. The other images were classified into different patterns (according of the physicist reporting); those with frequency varying from 2% to19% are shown in figure 2.

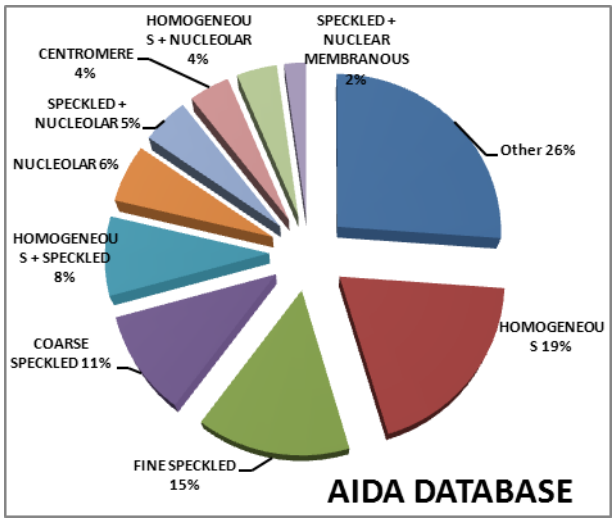

Fig. 2: Distribution of IIF patterns in the A.I.D.A. database (number of images 14393)

In a second step, 6974 images were anonymously exchanged between partners with respect of ethics and for blind double reporting by seniors or juniors immunologists. Young immunologists are PhD students (3) or fundamental immunologists (1).

2.4 CAD immune

The CAD used in the A.I.D.A. project is the *CyclopusCAD immuno* software, powered by CyclopusCAD srl, a spin-off of University of Palermo. It consists of an informatics program for image analysis using artificial intelligence [5-10].

2.5 Statistics calculation

The Accuracy and the Mean Class Accuracy (MAC) are adopted in this work as the measures of the performance.

Let CCR_k be the correct classification rate for class k determined as follows:

$$CCR_k = \frac{T_k}{N_k} \quad (1)$$

where T_k is the number of correct identifications of class k, while N_k is the total number of elements of class k. The Accuracy is defined by:

$$\text{Accuracy} = \frac{1}{k}\sum_k CCR_k \quad (2)$$

The MAC is determined by:

$$MAC = \frac{\sum_k CCR_k N_k}{\sum_k N_k} \quad (3)$$

The comparison of performance was carried out calculating the significance of the test using the probability (p) of the chi-square distribution between the expected value and the value obtained.

3 Results

In order to evaluate the level of concordance, a subsample of 589 wells (each with 3 images) reported by two senior immunologists, was analyzed. The results are shown in table 2 and revealed a level of concordance around 71%. The maximum of concordance has been obtained for Centromere pattern. The lower concordance level is observed with Fine Speckled pattern.

ISBN: 978-960-474-401-5

Table 2: Level of concordance between two senior immunologist readers of 589 wells

		Senior							
		NEG	HO	F_Sp	C_Sp	NU	CE	DOT	TOT
Senior	NEG	117	6	10	5	3			141
	HO	5	110	15	3	1			134
	F_Sp	26	22	49	24	1			122
	C_Sp	23	4	20	67				114
	NU	1				38			39
	CE						31		31
	DOT					1		7	8
	TOT	172	142	94	99	44	31	7	589

This level of concordance carries out the difficulty of interpretation of IIF images and the obvious need for a double reading and for Gold Standard.
For this aim, considering only images with double reporting concordance of 100%, a subsample database was extracted containing 1006 images representing all types of patterns (302 wells corresponding to 906 images) and including negative tests (100 images). In this Gold Standard database, the distribution of the different patterns is given in figure 3. All our approach is resumed in table 3.

	Homogen.	Fine_ speckled	Coarse_ Speckled	Nucleolar	Centromere	Nuclear_ DOT	TOT
GOLD	110	49	67	38	31	7	302 wells

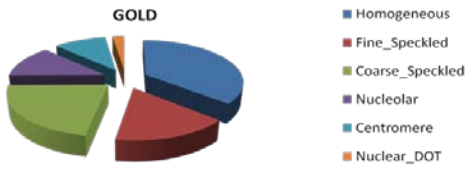

Fig. 3: Distribution of IIF patterns in the Gold Standard database (number of images 1006, number of wells 302)

Table 3: Number of IIF images in A.I.D.A. database: with one or two reports among which a Gold Standard sample with concordant reporting was extracted

	Images		
	with 1 Report	with 2 Reports	***Gold Standard***
Positive	12947	6274	906
Negative	1446	700	100
Total	**14393**	6974	1006

Our objective is to evaluate the added value of the CAD used as a second reader. We first tested CAD performance *stand alone* comparatively to junior immunologists. Four young fundamental immunologists (*juniors*) at the Faculty of Sciences of Tunis were involved as readers of images already reported by the experts (*seniors*). Before being compared to the CAD, the concordance of reporting of these young readers to a senior immunologist and between them has been analyzed. As shown in table 4, and as expected, the level of concordance between junior versus senior immunologists was lower than between seniors except for the reader 4 who is a more experienced fundamental immunologist but never involved in diagnosis.

Table 4: Percent of reporting concordance of junior readers *versus* senior or juniors readers.

Concordance % of Juniors *versus* Seniors				
Junior readers	***1***	***2***	***3***	***4***
Nb of wells	117	169	174	141
Concordance	37,6%	53,2%	42,5%	72,3%
Mean	45,8%		57,4%	
Concordance % of Junior *versus* Junior				
Juniors pair	***1 vs 2***		***3 vs 4***	
Nb of wells	265		219	
Concordance	46,8 %		68,5 %	

We also compared the young readers between them. We considered two pairs: juniors 1 and 2 on one hand and juniors 3 and 4 on the other. The results indicate, for each pair a concordance level that is near the mean of the concordance with a senior.
In another step, and excluding the junior reader 4, we have assessed the performance of two juniors reporting on Gold Standard wells (each with 3 images). Accuracy has been established considering intensity on one hand and patterns on the other.

ISBN: 978-960-474-401-5

We then compared this performance to that of the CAD which gave better results than young immunologists, as shown in tables 5 and 6.

Table 5: Comparison of CAD and junior reporting using gold standard images as reference

Parameters Readers	Intensity	Patterns	
	Accuracy	Accuracy	Mean accuracy
Junior 1	66,0%	48,0%	56,7%
Junior 2	66,0%	66,2%	64.2%
CAD	**85,5%**	**79,3%**	**79,4%**

Table 6: Accuracy and Mean Accuracy of CAD compared to gold standard used as reference.

CAD	HO	F_Sp	C_Sp	NU	CE	DOT	Other	ACC	MAC
HO	81.1%	9.5%	6.8%	2.7%	0.0%	0.0%	0.0%	79.3%	79.4%
F_Sp	6.5%	54.8%	16.1%	19.4%	3.2%	0.0%	0.0%		
C_Sp	0.0%	9.6%	80.8%	3.8%	3.8%	1.9%	0.0%		
NU	3.3%	10.0%	0.0%	86.7%	0.0%	0.0%	0.0%		
CE	3.4%	3.4%	0.0%	3.4%	89.7%	0.0%	0.0%		
DOT	0.0%	0.0%	16.7%	0.0%	0.0%	83.3%	0.0%		
Other	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%		

Compared with the two Readers, CyclopusCAD immune software showed higher Intensity Accuracy (vs Junior 1 p=0.016, vs Junior 2 p =0.016), higher Patterns Accuracy (vs Junior 1 p<0.0001, vs Junior 2 p =0.1) and higher MAC (vs Junior 1 p =0.0026, vs Junior 2 p =0.057).

In a last step we assessed the performance improvement of the young immunologist reports with the support of the CAD. The increase in accuracy is observed, in particular for the junior reader 1.

Table 7: Accuracy of junior reporting with the support of the CAD using Gold Standard images as reference

Parameters Readers	Intensity	Patterns	
	Accuracy	Accuracy	Mean accuracy
Junior 1	76,0%	69,5%	73,9%
Junior 2	66,0%	68,2%	66.3%

As regards the performance of Junior 1:
- Intensity Accuracy: varied from 66% to 76% showing an increase (p=0.21);
- Patterns Accuracy: varied from 48% to 69.5% and was significantly increased (p=0.002);
- MAC: varied from 56.7% to 73.9% and was significantly increased (p=0.02).

As regards the performance of the Junior 2:
- Intensity Accuracy: did not vary;
- Patterns Accuracy: varied from 66.2% to 68.2%, showing a low significance increase (p= 0.81)
- MAC: varied from 64.2% to 66.3, showing a low significance increase (p=0.79).

Conclusion

Before the end of its closure, the project A.I.D.A. is giving very encouraging preliminary results. First, the size of the database with around 14500 images accompanied with respective report is the biggest established in the world, in the field of Indirect ImmunoFluorescence applied to auto-immune diseases diagnosis. Second, around 1000 images with two concordant reports established by immunology experts and including different patterns constituted our first Gold Standard database that has been the basis for “learning” of the *CyclopusCAD immuno* software. This Gold Standard database is of great interest and has been considered as reference to evaluate young readers and CAD performance. We were able to demonstrate that the CAD is closer to the Gold Standard than junior immunologists.
Considering, the concordance level between senior immunologists evaluated at 71%, the CAD seems to bring the same level of performance. Indeed, when compared to Gold Standard, the CAD

ISBN: 978-960-474-401-5

showed a mean accuracy of 85% for intensity evaluation and 79% for pattern recognition.
Moreover the CAD support improves the performances of young readers up to 45%, as evidenced by the comparison of the Tables 5 and 7.
With these preliminary results, we can conclude that the objectives of the project are being reached with the demonstration that the CAD displays a higher performance than junior immunologists and equivalent results with immunology experts. In a further step, the Gold Standard database should be enriched, including more double reported and also triple reported images. With a high number of images of high quality reporting, we would be able to improve the CAD performance and our quality assessment approach in order to offer a product of high confidence to be used as second reader, to student learning and to diagnosis at distance.

Acknowledgments
We thank Dr. Marcella Zanetti for the excellent work and organizational support.
We thank the Department of Health of the Sicilian Region (Italy) and the Public Health Department of Tunis (Tunisia) for the support provided.

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This document was produced with the financial assistance of the European Union in the frame of the ENPI CT Italy-Tunisia 2007-2013. The views expressed herein can in no way be taken to reflect the official opinion of the European Union. Sole responsibility for the views, interpretations or conclusions contained in this document lies with the authors. Neither the European Commission nor Management Structures of the Program can be held responsible for the accuracy, completeness or use that may be made of the information contained herein.

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