Association between stressful life events and autoimmune diseases: A systematic review and meta-analysis of retrospective case-control studies

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Summary

Background Evidence of a relationship between stressful life events and the onset of autoimmune diseases is not univocal and there are no meta-analyses in the literature on the question.

Aim To look for differences in the number and type of stressful life events in the premorbid period between patients with autoimmune diseases and healthy subjects.

Method Review of the literature in PubMed and Scopus (January 1963 – May 2015).

Inclusion criteria We included retrospective case-control studies that compared patients diagnosed with autoimmune disorders and controls regarding the incidence of stressful events occurring before diagnosis, and that investigated said events with validated questionnaires.

Effect-size indexes By random-effect meta-analysis, two independent researchers calculated effect-size indexes as the difference between the means of the clinical groups and the control group in relation to the combined standard deviation.

Results The database searches produced 2490 articles, 14 of which were selected (3201 patients). Analysis showed a moderate but significant mean effect-size index [d=0.63, p<0.01], suggesting that autoimmune disorders are effectively associated with major stressful events in the premorbid period. The relationship between stressful events and autoimmune disease was weaker in studies with a high proportion of female subjects [β=-0.004, p<0.01] and stronger in studies that considered a longer interval between *stressors* and onset of disease [β=0.16, p<0.01].

Conclusions The results of this meta-analysis suggest that *stressors* may play an important role in the etiopathogenesis of autoimmune disorders. Only prospective studies can provide more certain inference about the causality of this relationship.

Key words: stress, stressful life events, autoimmune disease, meta-analysis.

Introduction

While autoimmune diseases differ clinically, epidemiologically and physiopathologically they have common pathogenetic mechanisms based on activation of B and/or T lymphocytes due to immune self-recognition. Etiopathogenesis is considered to be multifactorial; indeed, genetic, environmental and hormonal factors all contribute to their development, while physical and psychological stressors have also been implicated [1-7]. Studies on animal models and humans have demonstrated that stress affects immune responses through activation of the nervous and endocrine systems. In particular, the interaction between the activated neuroendocrine system and the immune system via hormonal mediators, neurotransmitters and cytokines could contribute to the development of autoimmune diseases [8-16].

Many studies have investigated the relation between stressful life events and development of autoimmune disorders. Most of the literature on the subject has been based on cross-sectional study design, in which stressful events in the premorbid period were assessed by self-rating questionnaires or semi-structured interviews [17-21]. In one retrospective cross-sectional case-control study conducted in the US, Linn et al. [17] investigated the role of stressful events in patients diagnosed with type I (autoimmune) and type II (non autoimmune) diabetes using the Holmes and Rahe Social Readjustment Scale [22]. They observed that the group of patients with type I diabetes reported a significantly higher number of stressful events in the 6 months before diagnosis than the group with type II diabetes and a control group of healthy subjects. In a study conducted in New Zealand, Stewart et al. [18] used the Schedule of Recent Experiences questionnaire [23] to compare scores obtained by a group of patients with rheumatoid arthritis, seronegative for rheumatoid factor, with a group of seropositive patients. The results showed that the second group had significantly higher scores than the first and than healthy controls. In a case-control study conducted in Croatia, Simonić et al. [21] used a questionnaire to study the frequency of stressful events during childhood and adolescence and found that patients diagnosed with psoriatic arthritis had higher scores than a control group without arthritis.

However, not all the studies confirmed a clear relation between stressors and autoimmune disease [24-27]. In one retrospective case-control study conducted in the UK, Carette et al. [25] investigated the possibility of differences between a group of patients with rheumatoid arthritis and a group of healthy subjects. The results did not show more stressful events before onset of symptoms in patients than in controls. Similarly, in Scandinavia, Hägglöf et al. [24] observed that a group of children with type I diabetes did not report significantly more stressful events in the year before onset than a control group of children of the same age. In a study in The Netherlands,

Strieder et al. [27] investigated the relation between stressful events in the last 12 months, detected by The Dutch Questionnaire on Recently Experienced Stressful Life Events [28], and positivity for thyroid peroxidase (TPO) antibodies in a large sample of women between 18 and 65 years of age. No significant differences in the number of stressful events were found between women positive and negative for TPO antibodies.

Thus the evidence currently available makes it impossible to determine whether there exists a relationship between autoimmune diseases and stressful events experienced prior to onset of symptoms. Rather, the literature provides a more coherent indication that stress is an exacerbating factor for these disorders [29-31]. Only one meta-analysis has summarised existing evidence on the role of stressors in a specific autoimmune disorder, multiple sclerosis [32]. Mohr et al. [32] analysed 14 correlational studies published in the period 1965-2003, identified by searching the electronic databases PubMed, PsycInfo and Psychological Abstracts, and showed a significant risk of exacerbation of autoimmune symptoms in patients who had experienced stressful events [d=0.53, IC 95%: 0.40-0.65, p<0.0001] compared to patients with the same disorder who had not suffered such events. However, this meta-analysis was restricted to a single autoimmune disease and the authors investigated the role of stress as a potential factor involved in exacerbation of symptoms, but they did not include studies that evaluated stressful events occurring in the premorbid period. Despite growing interest on the part of researchers for a possible association between stressful life events in the premorbid period and autoimmune diseases, current evidence in the literature does not seem to be univocal. A meta-analysis that considers a wide range of autoimmune diseases and provides a quantitative synthesis of the data so far available has yet to be conducted.

Aims

The objective of the present study was to summarise scientific evidence on the association between stressful life events and autoimmune diseases by a systematic review and meta-analysis. The specific aims were: a) to determine whether patients diagnosed with autoimmune diseases reported a significantly higher number of stressful life events in the premorbid period than healthy controls; b) to determine whether the relation between stressful events and autoimmune diseases is moderated by specific factors such as gender, interval (years) between stressors and diagnosis, and the type of tools used to measure stressful events (semi-structured interviews versus self-rating questionnaires). Finally, the relation between stressful events and autoimmune diseases was investigated comparing studies on systemic autoimmune diseases (rheumatoid arthritis, psoriatic arthritis, type I diabetes) with studies on organ-specific autoimmune diseases (Graves' disease and Hashimoto's thyroiditis).

Materials and methods

Meta-analysis protocol

In a previous phase of the study, the objectives, inclusion and exclusion criteria and statistical methods were described in a protocol that can be requested from the corresponding author. The protocol was drawn up according to PRISMA guidelines [33], adapted for meta-analysis of primary case-control studies.

Inclusion and exclusion criteria

The inclusion criteria regarded: a) type of study design; b) type of index cases and controls; c) type of measurements.

Study type and design. We only included retrospective case-control studies in which patients diagnosed with autoimmune diseases and controls were compared in relation to the incidence of stressful life events occurring in the period before diagnosis (premorbid period). A further inclusion criterion was that the study be published in a peer-reviewed journal. The date and the language in which the papers were written were not exclusion criteria.

Type of subjects. Only studies on humans were included, whether children and adolescents (under 16 years) or adults. Studies were included if conducted on patients diagnosed with autoimmune pathologies by standard diagnostic criteria (e.g. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). In particular, the criteria for definition of autoimmune diseases used for inclusion of studies were those proposed by Witebsky and Rose (1957), later revised by Rose (1993).

Regarding the type of control group, we included: studies in which the control subjects were healthy individuals undergoing diagnostic tests for autoimmune diseases that turned out to be negative; studies with subjects from the general population not undergoing tests; studies in which the controls were selected by matching of salient sociodemographic variables; studies in which controls were patients with diseases of psychosomatic etiology.

We excluded: studies in which differences in exposure to stressful events were only investigated among patients with different autoimmune diseases; studies in which patients with autoimmune diseases had comorbidities; studies conducted on patients with autoimmune diseases as well as psychiatric complaints, such as mood disorders.

Type of measurements. The stressful events were required to have been investigated by validated tools (semi-structured interviews or self-rating questionnaires) [34]. Such tools could have evaluated the frequency, number or perceived intensity of stressful events occurring in a specific period of time before onset of the disease. For the aims of the present meta-analysis, various types of environmental stressors, including major stressors and events with strong traumatic impact (loss of job, separation from spouse, bereavement) as well as stress linked to daily problems [35], were considered stressful life events. We excluded traumatic events associated with physical and/or sexual abuse in an intra- or extra-family setting. The timing of stressful events was not considered an exclusion criterion. Thus we included studies that investigated events occurring in childhood and adolescence, as well as those that investigated events occurring at a relatively more recent time (e.g. in the last years/months of the premorbid phase).

Study search procedure

The studies were identified by a systematic search in the electronic databases PubMed and Scopus (1st January 1963-31st May 2015) using the key words *stress, stressor, anguish, suffering, traumatic event* and *psychological* and linking each with the autoimmune-related key words *autoimmunity, autoimmune response, autoimmune disease*.

Study selection process

Two authors (BP and AP) independently selected the studies for meta-analysis in a three stage process based on title, abstract and the whole text. At the end of each stage, the two authors compared the studies excluded according to the predefined criteria. If they did not agree, the studies were included and evaluation was postponed to the next stage. If they did not agree after stage 3, another author (FF) was consulted as external assessor. In this way decisions about exclusion were settled by agreement between the three authors.

Evaluation of study quality

Two authors (FF and AP) independently evaluated the quality of each study by the Newcastle-Ottawa Quality Assessment Scale [36]. This tool assigns a maximum score of nine: four points regarding selection criteria for cases and controls (definition of cases, selection of cases, definition of controls, selection of controls), two points regarding the comparability criteria of cases and controls according to study design and statistical analysis (comparability in terms of age and in terms of gender) and three points for exposure verification criteria of cases and controls (exposure verification, same method of verification, no-response point). Studies scoring nine were classified

as high quality, those scoring seven or eight as medium quality, and those scoring less than seven as low quality. Disagreement in score attribution between the two authors was settled by discussion.

Meta-analysis method

Extraction of data and synthesis measures. Two authors (FF and AP) independently extracted and collected information regarding the following aspects from the selected studies: a) study design; b) study characteristics (data of publication, country in which it was conducted); c) characteristics of participants (gender, age, generational cohort, type of autoimmune disease, type of control group, sample size); d) type of stressful life event measurement tool (self-rating questionnaire or semi-structured interview). Any discrepancy between the information extracted by the two authors was discussed and settled in meetings held immediately after the data was entered on the spread sheets. To calculate the effect-size indexes, the two authors independently extracted the mean (and standard deviation) stressful event scores and data on the size of the groups of patients with autoimmune diseases and controls, from each of the selected studies. For studies that did not provide this information, the data used to calculate the effect-size indexes was obtained by the conversion formula of Ray et al. [37]. By this method it was possible to calculate the effect-size indexes for all included studies.

Calculation of effect-size indexes. The effect-size indexes were calculated with the formula (a) of Cohen [38]:

$$d = \frac{M_{CASE} - M_{CONTROL}}{SD_{COMBINED}} \qquad (a)$$

where M_{CASE} and $M_{CONTROL}$ are the means of the autoimmune and control groups, respectively, and $SD_{COMBINED}$ is the combined standard deviation.

The score of each index was weighted using the correction formula (b):

$$W_{zr} = 1/SE^2_{zr} \qquad (b)$$

where SE^2_{zr} is the standard error of the effect-size index calculated for each study.

Using the Cohen model, effect-size indexes greater than or equal to 0.80 were considered high, indexes in the range 0.80-0.50 moderate and indexes less than or equal to 0.20 of low. Meta-analysis was conducted by the random effect procedure, with significance set at <0.01.

Analysis of consistency. To evaluate variability among the studies we used two complementary indexes, the I^2 index [39] and Hedges Q [40], respectively. The former expresses the percentage of

heterogeneity of the effect-size indexes between studies [39]. A value of I^2 close to zero indicates homogeneity, whereas values of 25-50%, 50-75% and 75-100% indicate low, medium and high heterogeneity [39]. A significant value of Hedges Q suggests that variability among studies is greater that it would be if it were entirely due to sampling error [41]. We chose a significance level of <0.01.

Sensitivity analysis. To increase the internal consistency of the results regarding the relationship between stressful events and autoimmune disease, sensitivity analysis was conducted in a further phase. Evaluation of the effect-size indexes was restricted to case-control studies in which the control groups consisted exclusively of healthy subjects (n=12). The aim was to verify the consistency and improvement of the meta-analysis results with fewer studies, due to exclusion of those that used unwell subjects as controls.

Moderator coding. When consistency analysis suggested the presence of significant variability in the values of the effect-size indexes, we analysed the role of possible moderators by ANOVA with mixed effects models and single weighted least squares meta-regressions. The following characteristics were coded as moderator variables for the relation between stressful events and autoimmune diseases:

a) *Characteristics associated with participants:* gender of sample (coded as percentage of female gender);

b) *Timing of stressful events* coded as continuous variable, that is number of years between stressors and manifestation of autoimmune symptoms;

c) *Type of autoimmune disease:* comparison of systemic (rheumatoid arthritis, psoriatic arthritis, type I diabetes, multiple sclerosis) and organ-specific (Graves' disease, Hashimoto's thyroiditis);

d) *Type of measuring tool:* comparison of studies with self-rating questionnaires and those with semi-structured interviews.

In a meeting held after the independent codification by the moderators, no discrepancies in coding choices between the two authors emerged. For all analyses a significance level of <0.01 was used.

Publication bias. The effect of any publication bias was assessed by two complementary methods: the Classic Fail Safe Index N [42] and the Egger test statistic [43]. The former was obtained by computing the number of studies (N) not published necessary to make the p-value of overall effect-size index not significant, assuming that the single effect-size indexes of those studies was zero. The Egger test statistic is based on regression analysis in which the precision of each study is the

independent variable and the effect-size index divided by its standard error is the dependent variable (also called standard normal deviate on precision). A non significant result for the intercept of the regression test suggests that the hypothesis of publication bias can be rejected [43].

Results

Selection of studies

The search in the databases produced a total of 2490 articles, evaluated independently in a first phase by the two authors by reading the title. This led to exclusion of 2127 articles. Reading the abstract of the remaining 363 articles led to exclusion of a further 219. Reading the whole text of the remaining 144 articles (third phase) led to exclusion of 130 articles. Thirty-six were excluded because they were reviews of the literature, letters to editors, guidelines or declarations of consensus between experts. Seven articles were excluded because they concerned the relation between stressful events and exacerbation of autoimmune symptoms (e.g. studies in which the presence of stressors was investigated in the period after onset of the disease). Twenty-one articles were excluded because they were conducted on non-autoimmune diseases. Twelve articles were excluded because they regarded the relation between autoimmune diseases and psychological symptoms (e.g. depression, anxiety, burn out). Thirty-three articles were excluded because the authors had not used validated tools to measure the stressful events. Twenty-two articles were excluded for lack of sufficient data to calculate the effect-size index.

After this selection process, 14 studies (3201 patients) were included in the meta-analysis. The total number of effect-size indexes on the relation between stressful life events and autoimmune diseases was 30. The indexes resulted from the scales used to measure outcome (e.g. Coddington Questionnaire, Social Re-adjustment Rating Scale, Life Events and Difficulties Schedule, Health and Life Experience Questionnaire), which in many cases were the only measure used in the study, whereas in others, various measures were combined.

Figure 1 is a flow diagram showing the initial number of studies found in the database searches and the final number of studies included after the selection phases.

Descriptive characteristics of the selected studies

The 14 studies included in the meta-analysis were published in the period between 1983 [17] and 2012 [21]. Three studies were conducted in Asia or Oceania, namely China [44], Hong Kong [19] and New Zealand [18]. Ten studies were conducted in Europe, namely UK [25, 45-46], The Netherlands [27], Sweden [24, 47], Italy [48], Wales [49], Croatia [21] and Serbia [50]. One study was conducted in the US [17]. The design of all the selected studies was retrospective, cross-

sectional, case-control. Twelve studies involved adult populations and two concerned child populations [24, 47]. Two studies regarded patients diagnosed with multiple sclerosis [44, 46], five thyroid disease [19, 27, 48-50], three type I diabetes [17, 24, 47] and four rheumatoid or psoriatic arthritis [18, 21, 25, 45]. The meta-analysis therefore included nine studies on systemic and five on organ-specific autoimmune diseases.

The mean age of subjects was 40.7 years (*SD* 8.9; range 8.3-60.5 years). The percentage of female patients was 60.8 (*SD* 31.9; range 0-100). Mean sample number was 228.6 (*SD* 256.2; range 40-866). Five studies (35.7%) used semi-structured interviews to evaluate stressful events, whereas nine (64.3%) used self-rating questionnaires. The descriptive characteristics of the studies included in the meta-analysis is summarised in Table 1.

Evaluation of study quality

Four studies obtained a score of 9 (high quality), six of 7-8 (medium quality) and four <7 (low quality). Among the medium quality studies, one showed biased selection of controls, which were recruited among patients with other non autoimmune diseases; one did not specify whether controls had a history of autoimmune disorders. One study did not specify whether analysis of results took into account the effect of the factor age or if recruitment of controls was age-matched. Three studies did not specify whether the effect of the factor *sex* was checked or whether recruitment of controls was sex-matched. Two studies did not use objective methods of exposure assessment. Two studies reported wide differences in the response values between exposed and non exposed subjects. Among the low quality studies, three were *bias*ed in control selection, since controls were recruited among patients with other diseases, albeit not autoimmune. Three did not specify whether controls were without a history of autoimmune disorders. Three studies did not specify whether the effect of the factor *age* was considered in analysing the results or whether controls were age-matched with patients. Four studies did not say whether the effect of the factor *sex* was considered or whether controls were matched with patients for *sex*. Two studies reported large differences in response between exposed and non-exposed subjects. Table 2 provides a summary of the scores of the Newcastle-Ottawa Quality Assessment Scale for the various studies.

Differences in stressful life events between patients with autoimmune diseases and controls

The results showed a moderate but significant mean effect-size index (d=0.63, k=14, SE=0.20, IC 95%=0.23-1.03, p<0.01), suggesting that autoimmune pathology is associated with a significantly greater number of stressful life events in the period before diagnosis.

However, consistency analysis also showed strong heterogeneity among the selected studies ($Q_{(16)}$=368.31, I^2=95.65, p< 0.01), suggesting that the differences in *outcome* of the various studies may not be attributed exclusively to chance, but to other study-specific factors. The forest plot of Figure 2 shows the specific effect-size index of each study and the mean effect-size index regarding the differences in stressful events between groups of patients with autoimmune disorders and control groups.

Sensitivity analysis

In a later stage, we conducted sensitivity analysis on the effect-size indexes in order to check whether the relation between stressful events and autoimmune diseases remained significant after exclusion of two studies [21, 45] that included subjects with other illnesses in their control groups. The analysis enabled us to narrow the comparison down to control groups consisting exclusively of healthy subjects, with the aim of increasing the internal validity of the comparison. The results showed a large, statistically significant effect-size index (d=0.59, k=12, SE=0.16, IC 95%=0.27-0.92, I^2=95.10, p< 0.01), further confirming the hypothesis that subjects with autoimmune diseases report a significantly higher number of stressful events than healthy subjects, in the premorbid period.

Moderator analysis

This sub-meta-analysis investigated the role of female gender and timing of stressors as potential moderators of the relation between experience of stressful events and autoimmune diseases. Female gender showed a negative association with an effect of stressful events on autoimmune diseases (β=-0.004, k=13, IC 95%: -0.006-0.001, p<0.01), suggesting that in larger samples of female patients the relation between stressors and autoimmune diseases was of smaller magnitude. A meta-regression diagram of the effect-size indexes on female gender is shown in Figure 3.

The timing of the stressful events was coded as the length of the time interval (in years) in which the subjects experienced events whose impact was stressful. The results of the analysis showed that in studies in which a larger interval was considered, the relation between stressors and autoimmune diseases was stronger (β=0.16, k=12, IC 95%: 0.06-0.5, p<0.01). Figure 4 shows the meta-regression diagram of the effect-size indexes on the timing of stressful life events.

Mixed-effect ANOVA was then used to investigate differences in effect-size indexes between studies on patients with systemic autoimmune diseases and studies on patients with organ-specific autoimmune diseases. The results did not show any statistically significant differences between the two types of study ($Q_{(1)}$=2.09, p=0.14), so the relation between stressful events and autoimmune diseases did not turn out to be different between studies on systemic (d=0.37, k=23, SE=0.07, IC 95%: 0.23-0.51, p<0.01) and organ-specific pathologies (d=0.92, k=23, SE=0.37, IC 95%: 0.18-1.66, p< 0.01).

Finally, the same statistical procedure was used to conduct a post-hoc comparison on the relation between stressful events and autoimmune diseases between studies using self-rating questionnaires and studies relying on semi-structured interviews. The results did not reveal any statistically significant differences ($Q_{(1)}$=1.65, p=0.20). The relation between stressors and autoimmune diseases was associated with a moderate significant effect-size index for studies with self-rating (d=0.27, k=12, SE=0.09, IC 95%: 0.08-0.47, p<0.01). For studies with semi-structured interviews, though large, the effect was not significant and had a large confidence interval (d=1.38, k=5, SE=0.85, IC 95%: -0.29-3.06, p=0.10).

Table 3 summarises the effect-size indexes for all analyses, namely, overall analysis of the relation between stressful events and autoimmune diseases, moderator analysis (predictive effect of female gender, timing of stressors, type of tool used to measure stressful events and comparison between systemic and organ-specific autoimmune diseases) and sensitivity analysis conducted exclusively on control groups consisting of healthy subjects.

Publication bias

The effect of possible publication bias was evaluated by two complementary methods, namely Fail-safe N and Egger test statistic [42, 43]. The former was used to determine the number of unpublished studies. According to Rosenthal [42] it is possible to sustain that the results are not subject to publication bias if the number of studies necessary to make the overall effect-size index non significant exceeds 5k+10, where k is the number of studies included in the meta-analysis. The results produced a Fail-safe index N equal to 395 (Z=9.38, p<0.01), which means that 395 unpublished studies would be needed to bring the effect-size index within the non-significance interval.

The Egger test [43] showed a statistically non significant result for the intercept of the regression model (intercept=4.14, SE=1.96, 95% IC: -0.02-8.32, t=2.10, p=0.06). All together, the results of these tests showed an absence of publication bias, confirming the validity of the data included in the meta-analysis.

Discussion

The relation between stressful life events and onset of autoimmune diseases has become a subject of increasing interest for researchers.

In the present study we used meta-analysis techniques to obtain the first quantitative summary of the data currently available in the literature on this relationship with regard to stressors in the pre-morbid period. We included 14 studies published in peer-reviewed journals, conducted with retrospective case-control design, in which differences in stressful life events in the pre-morbid period were investigated in groups of patients with, and control subjects without, autoimmune diseases. Included studies comprised ones on patients with rheumatoid or psoriatic arthritis, type 1 diabetes, multiple sclerosis and autoimmune thyroid disease. A strong point of the meta-analysis was inclusion of studies from very different geographical/cultural settings: nine from Europe (five from northern Europe, two from eastern Europe, one from the Mediterranean area), three studies conducted in Asia or Oceania and one from America.

The results showed a significant relation between stressful life events in the pre-diagnostic period and development of autoimmune diseases, suggesting that stressors can play a major role in the etiopathogenesis of these diseases. This result cannot be ascribed to publication bias, which also seemed unlikely on the basis of the Egger statistical test [43]. The significant relation between stressful events and autoimmune diseases observed by us proved to be coherent with the literature on animal models and samples of human subjects [8-16], from which it emerged that stressful environmental factors tend to influence the immune system by activation of the nervous and endocrine systems.

The high variability of effect-size indexes observed led us to investigate the role of potential moderator factors in the relation between stressors and autoimmune diseases. The results of this analysis revealed that in samples comprising many female patients, the relation had a smaller amplitude, suggesting that for women stressful life events can have a more limited impact on the development of autoimmune diseases than in males. This is an interesting finding, considering the fact that the prevalence of systemic and organ-specific autoimmune diseases (especially autoimmune thyroiditis) is much higher in females. Since the pathogenesis of autoimmune diseases is multifactorial and certainly also includes sex-related hormonal factors, the component linked to stress could have less weight in women than in males.

The timing of stressful events proved to be another moderator variable of the relation between stressors and autoimmune diseases. In particular, the analysis showed that in studies that considered a larger interval for the premorbid period, the relation between stressors and

autoimmune diseases was associated with a greater effect, probably because the effect of stressful events increases with the interval over which they act.

Finally, the relation between stressful events and autoimmune diseases did not seem moderated by the type of disease, since no significant differences emerged between systemic and organ-specific pathologies.

Limits and implications for future research

Some limits of this meta-analysis should be underlined. A first aspect regards the fact that a relatively small number of studies were included, which suggests prudence in interpreting the results on the role of certain moderators, due to potential error of the second type. For example, the non significant difference between systemic and organ-specific autoimmune diseases could depend on the low statistical power of the test. For a correct evaluation of this possible bias it is therefore necessary to analyse a greater number of studies.

An aspect calling for great prudence in the interpretation of the results was the heterogeneity of the studies, since with sensitivity analysis, exclusion of studies in which controls were not healthy was unable to explain the heterogeneity of the data. Possible causes of heterogeneity could be the variety of stressful life events considered and therefore differences in the measuring tools used in the studies, that detect major stressors, such as marriage problems (separation, divorce) and work problems (job loss), and minor stressors, such as the hassles of daily life, for example physical, emotional or sexual abuse. Even the timing of stressful events could have a role in the development of diseases, suggesting that different stressors with different timing may have different impacts.

A further reason for heterogeneity could be the fact that different autoimmune diseases were included. The limited number of studies made it impossible to investigate the effect of specific stressful events or the effect of stressors on single diseases. Further studies into the interaction between type of stressful event and specific autoimmune pathologies are needed. Heterogeneity could also depend on the presence of four studies that used low quality research methods.

Incidentally, the heterogeneity of the scores of methodological quality obtained was noteworthy, especially the fact that of the 14 studies included, four had a high, six a medium and four a low quality score. The presence of low quality studies is a major limit. In these studies the effect of confounding variables such as age and gender was not controlled by matching recruited control subjects or by correction of the statistical analysis.

It is also necessary to consider certain methodological difficulties. A critical point regards the fact that the meta-analysis was conducted exclusively on studies with retrospective design, due to the small number of prospective studies in the literature. It is also possible that problems linked to the

memory of stressful events by subjects led to overestimation or underestimation of the impact of stressors. An alternative explanation of the results could be that patients could, for example, estimate the impact or frequency of the stressful events occurring before diagnosis or place them inaccurately with respect to the date of diagnosis. This effect could be linked to the fact that the efficiency of episodic memory may depend on the emotional state in which the subject finds himself/herself at the time of memory retrieval [51]. This memory distortion could be a major problem in subjects for whom the diagnosis of autoimmune disease is experienced as stressful. In fact, the long prodromal phase of autoimmune diseases, apparently asymptomatic or with few symptoms, could in itself be a cause of psychic discomfort and therefore stress. In any case, current detection tools suggest that stress can somehow be associated with autoimmune diseases or precede their manifestation.

One last limit regards the fact that observational studies were included. As underlined by Higgins and Greene [52], this type of design prevents to ascertain causal inferences about the relation between stressful events and development of autoimmune diseases. We recommend that prospective studies on the development of autoimmune diseases be conducted for a fixed period, starting from the stressful events, to confirm the evidence from retrospective observational studies.

Take-home messages

- Subjects with autoimmune diseases report a significantly higher number of stressful events than healthy subjects, in the premorbid period.*stressors*
- For women stressful life events can have a more limited impact on the development of autoimmune diseases than in males
- The timing of stressful events proved to be another moderator variable of the relation between stressors and autoimmune diseases
- The relation between stressful events and autoimmune diseases did not seem moderated by the type of disease
- We recommend that prospective studies be conducted to confirm the evidence from retrospective observational studies

Conflict of interest

The authors declare that they do not have any conflict of interests.

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Table 1. Descriptive characteristics of the selected studies (*n*=14).

	***n* (%)**	***M* (*SD*; range)**
Age of subjects		40.7 (8.9; 8.3-60.5)
Percentage of female subjects		60.84 (31.93; 0-100)
Sample size		228.64 (256.24; 40-866)
Type of autoimmune disease		
Thyroid disease	5 (35.71)	
Diabetes	3 (21.42)	
Arthritis	4 (28.57)	
Multiple sclerosis	2 (14.28)	
Tools used to measure stressful events		
Semi-structured interviews	5 (35.71)	
Self-rating questionnaires	9 (64.28)	
Generational cohort		
Adults	12 (85.71)	
Children or adolescents	2 (14.29)	
Study design		
Retrospective cross-sectional case-control	14 (100)	

Table 2. Indices of methodological quality of the studies according to the Newcastle-Ottawa Quality Assessment Scale for case control studies.

	Selection of subjects				**Comparability of subjects**		**Exposure**			**Total score**
Studies	Definition of cases	Representativity of cases	Selection of controls	Definition of controls	Comparability of first factor (age)	Comparability of additional factor (gender)	Presence/absence of exposure	Same method of determination	No response score	
Carette et al. 2000	Si	Si	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Conway et al. 1994	Yes	Yes	No	No	No	No	Yes	Yes	Yes	5
Grant et al. 1989	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Hagglof et al. 1991	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Kung 1995	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	7
Linn 1983	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	7
Liu et al. 2009	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	9
Oretti et al. 2003	Yes	Yes	No	Yes	Yes	No	Yes	Yes	No	6
Radosavljevic et al. 1996	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	8
Simonic et al. 2012	Yes	Yes	No	No	No	No	Yes	Yes	No	4
Sonino 1993	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	8
Stewart et al. 1994	Yes	Yes	Yes	No	No	No	Yes	Yes	Yes	6
Strieder et al. 2005	Yes	Yes	Yes	Yes	No	No	Yes	Yes	Yes	7
Thernlund et al. 1995	Yes	Yes	Yes	Yes	Yes	No	No	Yes	Yes	7

Table 3. Summary of effect-size indexes for the different analyses of the relationship between stressful life events and autoimmune diseases

	k	***d* (*p-value*)**	**Standard error**	**Standardized beta (*p-value*)**	**95% CI**	***I*²**	***Q* (*p-value*)**
Comparison of stressful events between patients with autoimmune diseases and control groups	14	0.63 (0.002)	0.20		0.23-1.03	95.65	368.31 (0.001)
Predictive effect of female gender on relation between stressful life events and autoimmune diseases	13		0.001	-0.004 (0.04)	-0.006-0.001		
Predictive effect of timing of stressors on relation between stressful life events and autoimmune diseases	12		0.05	0.16 (0.001)	0.060-0.250		
Comparison of relation between stressful events and autoimmune diseases in studies with self-rating questionnaires and studies with semi-structured interviews	14						1.65 (0.20)
Self-rating questionnaires	12	0.27 (0.005)	0.09		0.08-0.47		
Semi-structured interviews	5	1.38 (0.100)	0.85		-0.29-3.06		
Comparison of relation between stressful events and systemic or organ-specific autoimmune diseases							2.09 (0.14)
Systemic diseases	23	0.37 (0.001)	0.07		0.23-0.51		
Organ-specific diseases	7	0.92 (0.01)	0.37		0.18-1.66		
Sensitivity analysis only including studies with healthy control groups	12	0.59 (0.001)	0.16		0.27-0.92	95.10	388.01 (0.001)

Legend to figures

Figure 1. Flow diagram of study selection process.

Figure 2. Forest plot of size-effect indexes of the different studies and mean index in relation to differences in stressful events between groups of patients with autoimmune diseases and groups of controls (*n*=14).

Figure 3. Meta-regression of the size-effect indexes on the variable female gender (n=13).

Figure 4. Meta-regression of the size-effect indexes on the variable timing of stressful events (n=12).

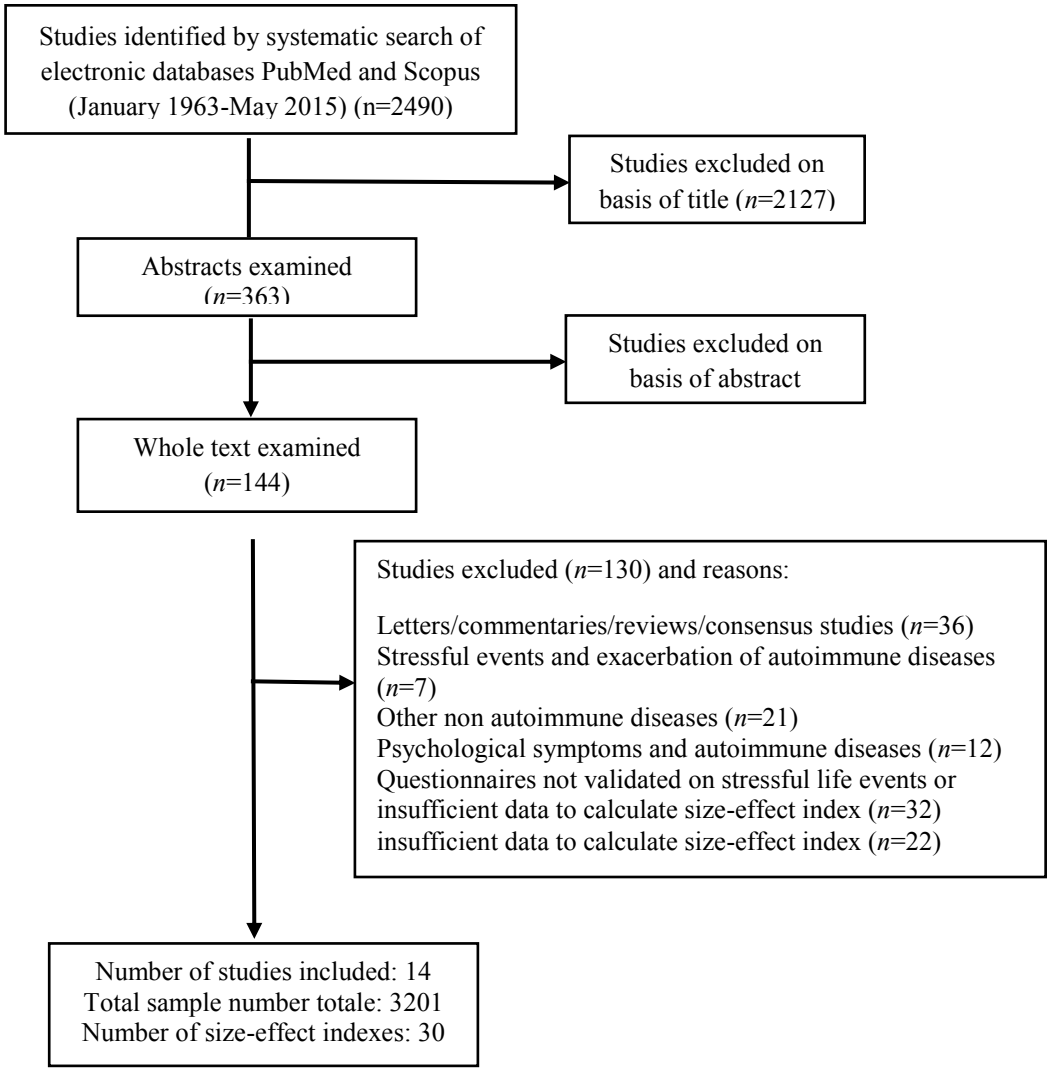

Studies identified by systematic search of electronic databases PubMed and Scopus (January 1963-May 2015) (n=2490)
Studies excluded on basis of title (n=2127)
Abstracts examined (n=363)
Studies excluded on basis of abstract
Whole text examined (n=144)
Studies excluded (n=130) and reasons:
Letters/commentaries/reviews/consensus studies (n=36)
Stressful events and exacerbation of autoimmune diseases (n=7)
Other non autoimmune diseases (n=21)
Psychological symptoms and autoimmune diseases (n=12)
Questionnaires not validated on stressful life events or insufficient data to calculate size-effect index (n=32)
insufficient data to calculate size-effect index (n=22)
Number of studies included: 14
Total sample number totale: 3201
Number of size-effect indexes: 30

Figure 1

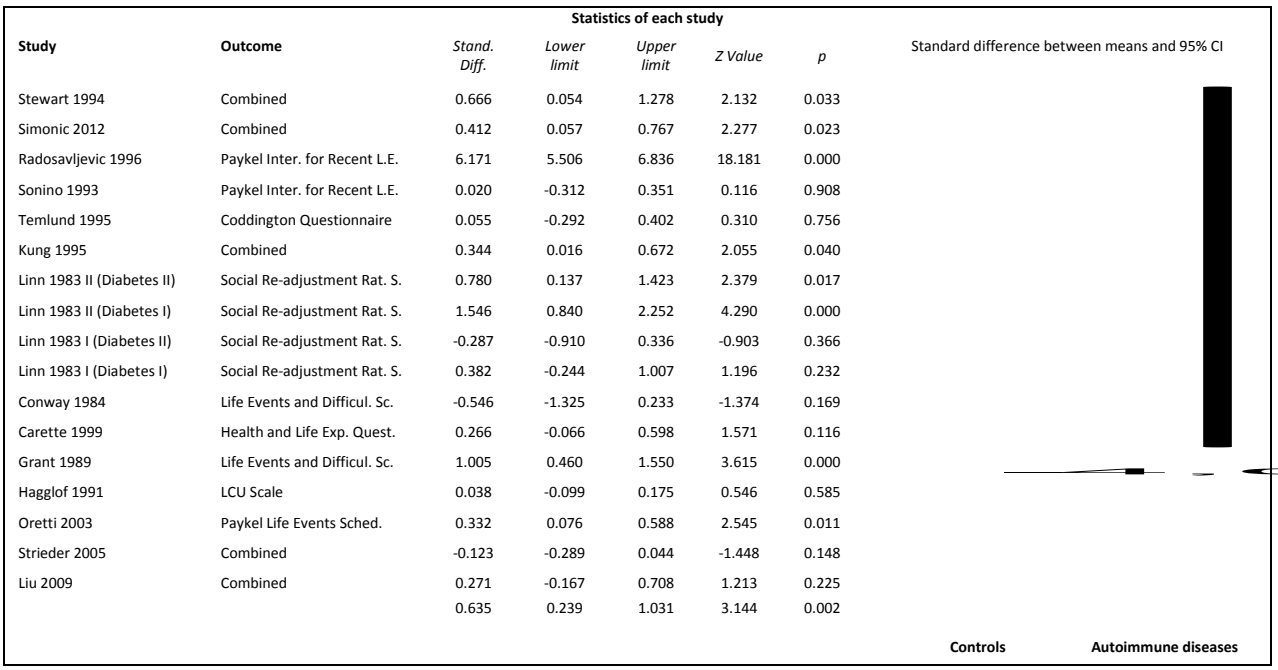


Figure 2

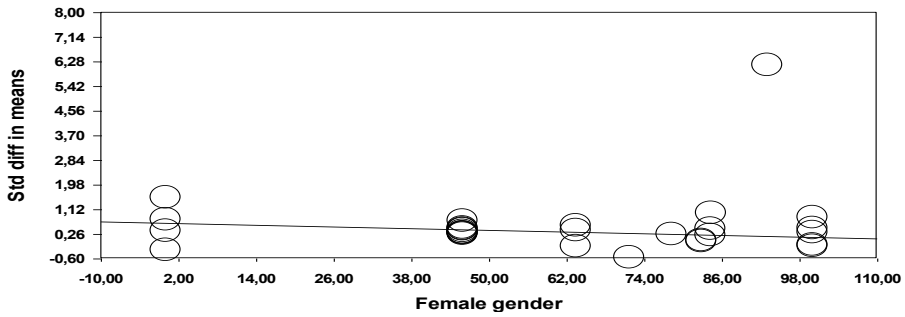
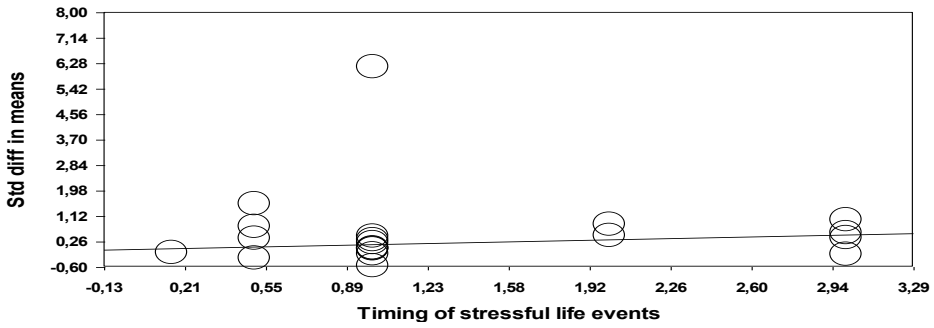


Figure 3


8,00
7,14
6,28
5,42
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Std diff in means
-0,13
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1,23
1,58
1,92
2,26
2,60
2,94
3,29
Timing of stressful life events

Figure 4