**EPSTEIN-BARR VIRUS IS THE CAUSE OF RHEUMATOID ARTHRITIS**

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**Abstract**

**Aim.** Many studies presented some evidence that EBV might play a role in the pathogenesis of rheumatoid arthritis. Still, there are conflicting reports concerning the existence of EBV in the synovial tissue of patients suffering from rheumatoid arthritis. This systematic review assesses the causal relationship between Epstein-Barr virus (EBV) and rheumatoid arthritis (RA) for gaining a better understanding of the pathogenesis of RA.

**Methods.** This systematic review and meta-analysis aim to answer among other questions the following: Is there a cause-effect relationship between Epstein-Barr virus and rheumatoid arthritis? The method of the conditio sine qua non relationship was used to proof the hypothesis without Epstein-Barr virus no rheumatoid arthritis. In other words, if rheumatoid arthritis is present, then Epstein-Barr virus has to be present too. The mathematical formula of the causal relationship k was used to proof the hypothesis, whether there is a cause-effect relationship between Epstein-Barr virus and rheumatoid arthritis. Significance was indicated by a p-value of less than 0.05.

**Results.** The studies analysed were able to provide convincing evidence that Epstein-Barr virus is a necessary condition (a conditio sine qua non) of rheumatoid arthritis. Furthermore, the studies analysed provide impressive evidence of a cause-effect relationship between Epstein-Barr virus and rheumatoid arthritis. **Conclusion.** EBV infection of human synovial tissues is a conditio sine qua non, a conditio per quam of rheumatoid arthritis. In other words, Epstein-Barr virus is the cause of rheumatoid arthritis.

**Keywords:** Epstein-Barr virus, rheumatoid arthritis, causal relationship

**INTRODUCTION**

Rheumatoid arthritis (RA), a systemic, predominantly (1) CD4+ T helper type 1 (Th1)-driven disease characterized by an extensive synovial hyperplasia and infiltration by macrophages, monocytes, lymphocytes and fibroblasts. Rheumatoid arthritis is a destructive, chronic and debilitating arthritis and can cause systemic complications. RA affects more or less about 1% of the world’s population (2). The prevalence of rheumatoid arthritis in men is twofold to fourfold less (3,4) than in women. The long-term prognosis of rheumatoid arthritis remains very poor. In particular, the average life expectancy of RA patients is reduced by 3 to 18 years (5). The direct costs of treatment of RA, the loss of employment and the indirect costs of disability due to RA are very high (6,7). At present there is no known cure for rheumatoid arthritis, an adequate use of various kinds of disease-modifying anti-rheumatic drugs may achieve complete remission in about 30-50% of RA patients. Many exposures investigated as possible risk factors for the development of rheumatoid arthritis such as dietary (antioxidants) factors (8) red meat protein (9), fat intake (10,11) breast feeding, the use of oral contraceptives or hormone replacement therapy (12) have shown no strong associations. Only cigarette smoking (13) has been found to increase the risk of rheumatoid arthritis. In the quest to uncover the unknown etiology of rheumatoid arthritis, viruses including Epstein-Barr virus (EBV), human herpesvirus-6, human herpesvirus-8, parvovirus (14) B19 (B19), HTLV-1, and human endogenous retroviruses-5 have all been hypothesized for many years to be involved in the pathogenesis of rheumatoid arthritis (15,16).

Epstein-Barr virus (EBV) is an ancient, ubiquitous virus determined by a 184 kbp-sized, double-stranded DNA genome which has infected probably more than 90% of the world’s population (17). Many studies presented some evidence suggesting
that especially EBV might play a role in the pathogenesis of RA. Among them Alspaugh and Tan (18-19) were one of the first. RA patients have higher levels of serum antibodies against EBV (20-24) than normal individuals. However, due to conflicting reports concerning the existence of EBV in the synovial tissue of RA patients a cause or the cause of rheumatoid arthritis remains unknown.

MATERIAL AND METHODS

RA is an autoimmune disease characterized by progressive and more or less persistent inflammation of joints of human body. At present, prognosis of RA may be very poor in the absence of an appropriate early treatment (25) with disease-modifying anti-rheumatic drugs (DMARDs) like methotrexate, sulphasalazine, azathioprine, antimalarials, gold-containing compounds, D-penicillamine and cyclosporin. In particular, an additional short-term duration treatment with corticosteroid is expected to prevent progressive course of RA with erosive joint damage and functional impairment.

FIGURE 1. Studies identification in search strategy. Adopted from PRISMA 2009 Flow Diagram (Moher26 et al., 2009; Liberati27 et al., 2009)

Statistical analysis

All statistical analyses were performed with Microsoft Excel version 14.0.7166.5000 (32-Bit) software (Microsoft GmbH, Munich, Germany). In order to increase the transparency, to correct some of the misprints of former publications and to simplify the understanding of this article several of the following lines are repeated sometimes word by word and taken from my former publications.

The 2x2 Table

The meaning of the abbreviations at, bt, ct, dt, Nt of the data table used are explained by a 2 by 2-table (Table 1).

| Condition At (risk factor) | Condition Bt (outcome) | total |
|---------------------------|------------------------|-------|
| Yes = +1                  | at                     | At    |
| Not = +0                  | ct                     | dt    |
| total                     | Bt                     | Bt    |

In general it is \((a+b) = A_t, (c+d) = A_t, (a+c) = B_t, (b+d) = B_t\) and \(a+b+c+d = N_t\). Equally, it is \(B_t = A_t + A_t = N_t\). In this context, it is \(p(a) = p(A_t \cap B_t)\), \(p(A_t) = p(at)+p(bt)\) or \(p(A_t) = p(A_t \cap C_t) + p(bt)\) = \(p(A_t \cap C_t) + p(A_t \cap C_t)\) while \(p(at)\) is not defined as \(p(at)\). In the same context, it is \(p(B_t) = p(at)+p(c) = p(A_t \cap C_t) + p(ct)\) and equally in the same respect \(p(B_t) = 1-p(B_t) = p(bt)+p(dt)\).

Furthermore, the joint probability of At and Bt is denoted in general by \(p(A_t \cap B_t)\). Thus far, it is \(p(A_t \cap C_t) = p(A_t) - p(bt) = p(B_t) - p(ct)\) or in other words it follows clearly that \(p(B_t) + p(bt) - p(ct) = p(at)\). In general, it is \(p(at)+p(c)+p(bt)+p(dt) = 1\).

The data of the studies analysed

The data of the studies analysed are presented by several tables (Table 2, Table 4, Table 6, Table 7, Table 8, Table 9, Table 10, Table 11). The meaning of the abbreviations at, bt, ct, dt, Nt of tables is explained by a 2 by 2-table (Table 1) too. Some studies provided self-contradictory data (Table 3, Table 5) and were not considered for a re-analysis.

Independence

In the case of independence of At and Bt it is generally valid that

\[
p(A_t \cap B_t) = p(A_t) \times p(B_t)
\]

(1)

Exclusion (At Excludes Bt and Vice Versa Relationship)

The mathematical formula of the exclusion relationship (28-48) (At excludes Bt and vice versa) of a population was defined as

\[
p(A_t \mid B_t) = \frac{b_t + c_t + d_t}{N_t}
\]

\(= 1 - p(a_t)\)

\(= p(b_t) + p(c_t) + p(d_t)\)

\(= p(c_t) + (1 - p(B_t))\)

\(= p(b_t) + (1 - p(A_t))\)

\(= +1\)

(2)

and used to proof the hypothesis: At excludes Bt and vice versa.
### TABLE 2. Without EBV VCA IgG positivity no RA.

| Study Id | Year | Country | Risk Factor | Case_P | Case_T | Con_P | Con_T | k   | p-val  | X²(SINE) | X²(IMP) | X²(IMP*SINE) | X²(EXCL) |
|----------|------|---------|-------------|--------|--------|-------|-------|-----|--------|----------|---------|---------------|---------|
| Ng et al. | 1980 | UK      | EBV VCA IgG | 59     | 64     | 41    | 50    | 0.15 | 0.0611335 | 16.40    | 16.72   | 87.70         |
| Ferrell et al. | 1981 | USA     | EBV VCA IgG | 76     | 80     | 45    | 51    | 0.12 | 0.0987540 | 16.37    | 16.52   | 118.36        |
| Venables et al. | 1985 | UK      | EBV VCA IgG | 37     | 38     | 23    | 26    | 0.18 | 0.1554984 | 8.44     | 8.44    | 57.26         |
| Yao et al. | 1986 | UK      | EBV VCA IgG | 31     | 33     | 24    | 26    | 0.03 | 0.3770384 | 10.04    | 10.11   | 45.10         |
| Shirodaria et al. | 1987 | UK      | EBV VCA IgG | 26     | 26     | 24    | 26    | 0.2  | 0.2450980 | 11.05    | 11.05   | 38.01         |
| Youinou et al. | 1992 | France  | EBV VCA IgG | 98     | 100    | 49    | 50    | 0.00 | 0.4489387 | 16.00    | 16.02   | 159.73        |
| Blashke et al. | 2000 | Germany | EBV VCA IgG | 55     | 55     | 53    | 60    | 0.24 | 0.0088147 | 25.52    | 25.53   | 81.51         |
| Us et al. | 2011 | Turkey  | EBV VCA IgG | 85     | 85     | 48    | 50    | 0.16 | 0.1354339 | 16.66    | 16.97   | 137.69        |
| Sherina et al., 2017 | 2017 | Sweden  | EBV VCA IgG | 970    | 987    | 679   | 700   | 0.04 | 0.0294958 | 279.18   | 279.45  | 1522.31       |

Total: 1437, 1468, 986, 1039, X² Calculated (SINE) = 0.8597

Alpha = 0.05

Degrees of freedom (d.f.) = 9

X² Critical (SINE) = 16.919

When using data to perform some analysis, several conditions must be taken into consideration. Unfortunately, not all data are appropriate for detailed analysis. Due to formal mathematical requirements it is possible to identify data as self-contradictory and it is necessary to exclude these data from further analysis. The reason for the self-contradiction of the data is marked in bold numbers/letters. These studies were not considered for further analysis even if all these studies supported the hypothesis without EBV VCA IgG sero-positivity no RA. The term #DIV/0! denote the case that there is a division by zero.

### TABLE 3. EBV VCA IgG self-contradictory data, not considered for a meta-analysis.

| Study Id | Year | Country | Risk Factor | Case_P | Case_T | Con_P | Con_T | k   | X²(SINE) | X²(IMP) | X²(IMP*SINE) | X²(EXCL) |
|----------|------|---------|-------------|--------|--------|-------|-------|-----|----------|---------|---------------|---------|
| Phillips et al. | 1976 | USA     | EBV VCA IgG | 31     | 33     | 32    | 33    | -0.0727393 | 0.07    | 15.75         | 15.82   | 42.96         |
| Nakabayshi | 1981 | Japan   | EBV VCA IgG | 32     | 32     | 15    | 15    | #DIV/0!    | 0.01    | 4.47          | 4.48    | 52.12         |
| Venables et al. | 1981 | UK      | EBV VCA IgG | 94     | 100    | 32    | 33    | -0.0574427 | 0.30    | 7.88          | 8.18    | 156.81        |
| Musiani et al. | 1987 | Italy   | EBV VCA IgG | 35     | 35     | 40    | 40    | #DIV/0!    | 0.01    | 20.80         | 20.81   | 49.88         |
| Zhang et al. | 1993 | Finland | EBV VCA IgG | 50     | 50     | 49    | 49    | #DIV/0!    | 0.01    | 23.76         | 23.77   | 73.76         |
| Mousavie-Jazi et al. | 1998 | Sweden  | EBV VCA IgG | 27     | 28     | 12    | 12    | -0.1048284 | 0.01    | 3.39          | 3.40    | 43.09         |
| Zhang et al. | 1999 | China   | EBV VCA IgG | 75     | 91     | 38    | 45    | -0.02544181 | 2.64    | 12.44         | 15.08   | 110.11        |
| Jorgensen et al. | 2008 | Denmark | EBV VCA IgG | 31     | 33     | 238   | 245   | -0.0585413 | 0.07    | 209.69        | 209.76  | 31.65         |
| Lünemann et al. | 2008 | USA     | EBV VCA IgG | 25     | 25     | 20    | 20    | #DIV/0!    | 0.01    | 8.45          | 8.46    | 37.35         |

Total: 400, 427, 476, 492
TABLE 4. Without EBV EBNA IgG positivity no RA.

| Study Id | Year | Country | Risk Factor | Case_P | Case_T | Con_P | Con_T | k   | p-val | $X^2$(SINE) | $X^2$(IMP) | $X^2$(IMP^SINE) | $X^2$(EXCL) |
|----------|------|---------|-------------|--------|--------|-------|-------|-----|-------|-------------|------------|----------------|------------|
| Ferrell et al. | 1981 | USA     | EBV EBNA-1 IgG | 83     | 83     | 47    | 53    | 0.26884692 | 0.002921342 | 0.00 | 16.63      | 16.64      | 134.36        |
| Shirodaria et al. | 1987 | UK      | EBV EBNA-1 IgG | 23     | 26     | 21    | 26    | 0.10660036 | 0.227268212 | 0.24 | 9.55       | 9.79       | 30.98         |
| Youinou et al. | 1992 | France  | EBV EBNA-1 IgG | 90     | 100    | 41    | 50    | 0.11338681 | 0.078226412 | 0.90 | 12.52      | 13.42      | 141.25        |
| Mousavi-Jazi et al. | 1998 | Sweden  | EBV EBNA-1 IgG | 27     | 28     | 10    | 12    | 0.22783558 | 0.187044534 | 0.01 | 2.44       | 2.45       | 44.06         |
| Blashke et al. | 2000 | Germany | EBV EBNA-1 IgG | 48     | 55     | 51    | 60    | 0.03280399 | 0.200258806 | 0.77 | 25.76      | 26.53      | 63.81         |
| Lünemann et al. | 2008 | USA     | EBV EBNA-1 IgG | 21     | 25     | 16    | 20    | 0.05198752 | 0.284334686 | 0.49 | 6.49       | 6.98       | 28.17         |
| Erre et al. | 2015 | Italy   | EBV EBNA-1 IgG | 69     | 77     | 40    | 58    | 0.25916219 | 0.002049224 | 0.73 | 14.31      | 15.04      | 103.99        |

Total 361 394 226 279 3.1435

Alpha = 0.05
Degrees of freedom (d.f.) = 7
$X^2$ Critical (SINE) = 14.0671
$X^2$ Calculated (SINE) = 3.1435
Case_P: cases, positive; Case_T: cases, total; Con_P: controls, positive; Con_T: controls, total.

TABLE 5. EBV EBNA IgG self-contradictory data, not considered for a meta-analysis.

| Study Id | Year | Country | Risk Factor | Case_P | Case_T | Con_P | Con_T | k   | $X^2$(SINE) | $X^2$(IMP) | $X^2$(IMP^SINE) | $X^2$(EXCL) |
|----------|------|---------|-------------|--------|--------|-------|-------|-----|-------------|------------|----------------|------------|
| Sculley 1986 Australia EBV EBNA-1 IgG | 49     | 72     | 41    | 49    | -0.175625 | 7.03 | 18.23 | 25.26 | 58.81       |
| Musiani et al. 1987 Italy EBV EBNA-1 IgG | 35     | 35     | 40    | 40    | #DIV/0! | 0.01 | 20.80 | 20.81 | 49.88       |
| Davis et al. 1999 Australia EBV EBNA-1 IgG | 39     | 50     | 35    | 43    | -0.04198663 | 2.21 | 16.08 | 18.29 | 49.68       |
| Jorgensen et al. 2008 Denmark EBV EBNA-1 IgG | 29     | 33     | 231   | 245   | -0.08421061 | 0.37 | 204.35 | 204.72 | 27.74       |
| Us et al. 2011 Turkey EBV EBNA-1 IgG | 85     | 87     | 50    | 50    | -0.092273  | 0.03 | 18.15 | 18.18 | 134.96      |
| Yazbek et al. 2011 Brazil EBV EBNA-1 IgG | 127    | 140    | 130   | 143   | -0.00337194 | 1.12 | 65.25 | 66.37 | 176.57      |

Total 315 345 486 521

The reason for the self-contradiction of the data above is marked in bold numbers/letters. These studies were not considered for further analysis even if most of these studies supported the hypothesis without EBV EBNA IgG sero-positivity no RA. #DIV/0! denotes the case that there is a division by zero.
**Necessary Condition (Conditio Sine Qua Non)**

The mathematical formula of the necessary condition relationship (28-48) (conditio sine qua non) of a population was defined as

\[
p(A_i \leftarrow B_i) = \frac{a_i + b_i + d_i}{N_i} = p(a_i) + p(b_i) + p(d_i) = p(a_i) + (1 - p(B_i)) = 1
\]

and used to proof the hypothesis: without At no Bt.

**Sufficient Condition (Conditio per Quam)**

The mathematical formula of the sufficient condition relationship (28-48) (conditio per quam) of a population was defined as

\[
p(A_i \rightarrow B_i) = \frac{a_i + c_i + d_i}{N_i} = p(a_i) + p(c_i) + p(d_i) = p(d_i) + p(B_i) = 1
\]

and used to prove the hypothesis: if At then Bt.

**The X² Goodness of Fit Test of a Necessar**

Under conditions where the chi-square goodness (8-48) of fit test cannot be used it is possible to use an approximate and conservative (one sided) confidence interval known as the rule of three. Using the continuity correction, the chi-square value of a condition sine qua non distribution before changes to

\[
\chi^2 = \left( \frac{c_i - \left( \frac{1}{2} \right)}{B_i} \right)^2 + 0 = 0
\]

**The X² Goodness of Fit Test of the Exclusion Relationship**

The chi square value with degree of freedom 2-1=1 of the exclusion relationship (28-48) with a continuity correction can be calculated as

\[
\chi^2 (EXCL) = \frac{(-a_i - 0.5)^2}{A_t} + \frac{(-a_i - 0.5)^2}{B_t}
\]

The chi square Goodness of Fit Test of the exclusion relationship examines how well observed data are compared with the expected theoretical distribution of an exclusion relationship.

**The Mathematical Formula of the Causal Relationship k**

The mathematical formula of the causal relationship (28-48) k is defined at every single event, at every single Bernoulli trial t, as

\[
k(A_i, B_i) = \frac{p(A_i \cap B_i) - (p(A_i) \times p(B_i))}{\sqrt{(p(A_i) \times p(A_i)) \times (p(B_i) \times p(B_i))}}
\]

where At denotes the cause and Bt denotes the effect. The chi-square distribution can be applied to determine the significance of causal relationship k. Pearson’s (49) concept of correlation (50) is not identical with causation (28,36,37). Causation as such is not identical with correlation. This has been proven many times and is widely discussed in many publications (51).

**The 95% Confidence Interval of the Causal Relationship k**

A confidence interval (CI) of the causal relationship k calculated from the statistics of the observed

| Study Id | Year | Country  | Risk Factor | Case_P | Case_T | Con_P | Con_T | k     | p-val | X²(SINE) | X²(IMP) | X²(IMP*SINE) | X²(EXCL) |
|----------|------|----------|-------------|--------|--------|-------|-------|-------|-------|----------|---------|---------------|---------|
| Mousavie-Jazi et al. | 1998 | Sweden   | EBV PCR DNA | 2      | 31     | 0     | 14    | 0.1449318 | 0.46996997 | 26.20    | 0.13     | 26.33         | 1.20    |
| Saal et al. | 1999 | Germany  | EBV PCR DNA | 29     | 84     | 8     | 81    | 0.2954235 | 9.29228E-05 | 35.36    | 1.52     | 36.88         | 31.62   |
| Takeda et al. | 2000 | Japan    | EBV PCR DNA | 15     | 32     | 0     | 30    | 0.5469937 | 6.07959E-06 | 8.51     | 0.02     | 8.52          | 20.59   |
| Chiu et al. | 2013 | Taiwan   | EBV ISH    | 23     | 23     | 0     | 13    | 1     | 4.32753E-10 | 0.01    | 0.01        | 0.02    | 44.02        |
| Erre et al. | 2015 | Italy    | EBV PCR DNA PBMC | 61 | 77 | 33 | 58 | 0.2403144 | 0.00322558 | 3.12    | 11.24    | 14.36         | 86.47   |

**TABLE 6. EBV PCR DNA and ISH studies and RA.**
data can help to estimate the true value of an unknown population parameter with a certain probability. Under some conditions, the 95% interval for the causal relationship $k$ is derived (47) as

$$\left\{ k(A_1, B_1) - \frac{5}{\sqrt{n}}, k(A_1, B_1) + \frac{5}{\sqrt{n}} \right\}$$  \hspace{1cm} (8)$$

Hypergeometric distribution

The hypergeometric distribution with its own and very long history (52,53,54,55) is defined by the parameters population size, event count in population, sample size and can be used to calculate the exact probability of an event even for small samples which are drawn from relatively small populations, without replacement.

The hypergeometric distribution differs from the binomial distribution. In contrast to the hypergeometric distribution, the probability of a binomially distributed random variable is the same from trial to trial.

The probability of having exactly at (Table 1) successes or the significance of the causal relationship $k$ can be tested under conditions of sampling without replacement by the hypergeometric distribution (56) as

$$p(a_i) = \frac{\binom{A_i}{a_i} \times \binom{N_i - A_i}{B_i - a_i}}{\binom{N_i}{B_i}}$$  \hspace{1cm} (9)$$

**TABLE 7. The parvovirus B19 Study of Sherina et al., 2017**

| RA <B> | Yes | No | Total |
|--------|-----|----|-------|
| B19 IgG Yes | 742 | 504 | 1246 |
| No | 237 | 188 | 425 |
| Total | 979 | 692 | 1671 |

$$k = +0.0335$$

$$p \text{ value (k)} = 0.017813306$$

$$95\% \text{ CI (k)} = (-0.0212;0.0882)$$

**TABLE 8. The CMV Study of Sherina et al., 2017**

| RA <B> | Yes | No | Total |
|--------|-----|----|-------|
| CMV IgG Yes | 713 | 531 | 1244 |
| No | 274 | 169 | 443 |
| Total | 987 | 700 | 1687 |

$$k = -0.0405$$

$$p \text{ value (k)} = 0.011242387$$

$$95\% \text{ CI (k)} = (-0.0139;0.0950)$$

**Odds Ratio**

The odds ratio (OR) is given by

$$\text{OR}(A_1, B_1) = \frac{a_i}{b_i} / \frac{c_i}{d_i} = \frac{a_i \times d_i}{c_i \times b_i} \hspace{1cm} (10)$$

It is necessary to point to the case were $ct=0$. Under conditions were $ct=0$, there is a conditio sine qua non relationship between $At$ and $Bt$ while the Odds ratio collapses. To date, it is not generally accepted to divide by zero.

The Odds ratio cannot speak about the natural, profound and far reaching conditio sine qua non relationship but must pass over in silence on this relationship. Pagano & Gauvreau (60) are quietly returning through the back door to circumvent this fundamental problem of Odds ratio by adding (60) 0.5 to the cells at, $bt$, $ct$, $dt$.

This simple way to circumvent the inconsistency and spectacular methodological incompleteness of Odds ratio is fundamentally misleading. To date, a substantial amount of research is analyzed by the Odds ratio. The more serious difficulty of this point of view is that it appears to be impossible to rely on Odds ratio in principle.

Furthermore, under conditions were $bt=0$, a conditio per quam relationship between $At$ and $Bt$ is given while the Odds ratio collapses again.

For this reason, the Odds ratio is overshadowed by a deep theoretical inconsistency and appears not to be grounded on a seemingly sound piece of reasoning.
More likely, the Odds ratio (OR) is nothing more but Yule’s coefficient of association (6) re-written (62) in a non-normalized form and given by:

\[
Q(A_i, B_i) = \frac{\text{OR}(A_i, B_i) - 1}{\text{OR}(A_i, B_i) + 1} = \frac{(a_i \times d_i) - (b_i \times c_i)}{(a_i \times d_i) + (b_i \times c_i)}
\]

(11)

Under conditions where Yule’s coefficient of association (Yule, 1900) \( Q = 0 \), there is no association. Although severely and justifiably criticized especially by Karl Pearson (1857–1925), the long-time and rarely challenged leader of statistical science and Heron (63), Odds ratio is still regularly referred to. The standard error and 95% confidence interval of the Odds ratio (OR) can be calculated according to Altman (64). Given the severely limited character of odds ratio, the standard error of the log Odds ratio is calculated as:

\[
\text{SE}\left(\ln(\text{OR}(A_i, B_i))\right) = \sqrt{\frac{1}{a_i} + \frac{1}{b_i} + \frac{1}{c_i} + \frac{1}{d_i}}
\]

(12)

where \( \ln \) denotes the logarithmus naturalis. The 95% confidence interval of the odds ratio is given by:

\[
\exp\left[\ln(\text{OR}(A_i, B_i)) - 1.96 \times \text{SE}\left(\ln(\text{OR}(A_i, B_i))\right)\right]
\]

to

\[
\exp\left[\ln(\text{OR}(A_i, B_i)) + 1.96 \times \text{SE}\left(\ln(\text{OR}(A_i, B_i))\right)\right]
\]

(13)

**TABLE 9. The EBV Study of Sherina et al., 2017**

| RA <B> | Yes | No | Total |
|--------|-----|----|-------|
| EBV VCA IgG <A> | Yes | 970 | 679 | 1649 |
| No | 17 | 21 | 38 |
| Total | 987 | 700 | 1687 |

\[ k = +0.0424 \]

\[ p \text{ value} (k) = 0.29495888 \]

95% CI (k) = (-0.0120; 0.0969)

Odds ratio = 1.7647

95% CI (Odds ratio) = (0.9241; 3.3700)

**TABLE 10. The Study of Saal et al.**

| RA <B> | Yes | No | Total |
|--------|-----|----|-------|
| EBV PCR DNA <A> | Yes | 29 | 8 | 37 |
| No | 55 | 73 | 128 |
| Total | 84 | 81 | 165 |

\[ k = +0.2954 \]

\[ p \text{ value} (k) = 9.29228E-05 \]

95% CI (k) = (0.1213;0.4695)

Odds ratio = 4.8114

95% CI (Odds ratio) = (2.0413; 11.3405)

**TABLE 11. The Study of Takeda et al.**

| RA <B> | Yes | No | Total |
|--------|-----|----|-------|
| EBV PCR DNA <A> | Yes | 15 | 0 | 15 |
| No | 17 | 30 | 47 |
| Total | 32 | 30 | 62 |

\[ k = +0.5470 \]

\[ p \text{ value} (k) = 6.07959E-06 \]

95% CI (k) = (0.2630; 0.8310)

\[ \text{IF } <A> \text{ THEN } <B> \]

\[ p \text{ (IMP)} = 1.0000 \]

\[ \chi^2 \text{ (IMP)} = 0.0167 \]

The unknown population proportion pupper

Tests of hypotheses concerning the sampling distribution of the sample proportion \( p \) (i. e. conditio sine qua non p(SINE), conditio per quam p(IMP) et cetera) can be performed using the normal approximation. The calculation of the rejection region based on the sample proportion to construct a confidence interval for an unknown population propor-
tion pupper can be performed under conditions of sampling without replacement by the formula

\[
\pi_{\text{critical upper}} = p \left(1 - \frac{1}{2 \times n}\right) - \left(Z \times \sqrt{\frac{p(1-p)}{n}} \times \left(\frac{N-n}{N-1}\right)\right)
\]

(14)

while the term \(\left(\frac{N-n}{N-1}\right)\) denotes the finite population correction (67).

**TABLE 12. The Study of Chiu et al.**

|               | RA <B> |   |   |   |
|---------------|--------|---|---|---|
| EBV ISH <A>   |        |   |   |   |
| Yes           | 23     | 0 | 23|
| No            | 0      | 13| 13|
| Total         | 23     | 13| 36|

\[k = \pm 1.0000\]

\[p\text{ value } (k) = 4.32753E-10\]

**WITHOUT <A>, NO <B>**

\[p (\text{SINE}) = 1.0000\]

\[X^2 (\text{SINE}) = 0.0109\]

**IF <A>, THEN <B>**

\[p (\text{IMP}) = 1.0000\]

\[X^2 (\text{IMP}) = 0.0109\]

\[<A>\text{ is SINE and IMP of <B>}\]

\[p(\text{SINE} \land \text{IMP}) = 1.0000\]

\[X^2 (\text{SINE} \land \text{IMP}) = 0.0217\]

**The Chi Square Distribution**

The following critical values (65,66) of the chi square distribution (68) as visualized by Table 13 are used in this publication.

**TABLE 13. The critical values of the chi square distribution (degrees of freedom: 1)**

| p-Value          | One sided \(X^2\) | Two sided \(X^2\) |
|------------------|-------------------|------------------|
| 0.10000000000    | 1.642374415       | 2.705543454      |
| 0.05000000000    | 2.705543454       | 3.841458821      |
| 0.04000000000    | 3.06490172        | 4.217845888      |
| 0.03000000000    | 3.537384596       | 4.70922247       |
| 0.02000000000    | 4.217845888       | 5.411894311      |
| 0.01000000000    | 5.141894341       | 6.338966011      |
| 0.00100000000    | 9.549535706       | 10.82756617      |
| 0.00010000000    | 13.831083622      | 15.13670523      |
| 0.00001000000    | 18.189293483      | 19.51142096      |
| 0.00000100000    | 22.59504266       | 23.92812698      |
| 0.00000010000    | 27.03311129       | 28.37398736      |
| 0.00000001000    | 31.49435797       | 32.84125335      |
| 0.00000000100    | 35.97368894       | 37.32489311      |
| 0.00000000001    | 40.46665791       | 41.82145620      |

**The rule of three**

The Chi-square goodness of fit test (68) used to test whether a sample distribution is identical with a theoretical distribution yields only an approximate p-value and works when the dataset analyzed is large enough (n ~ 30 and more). An approximate and conservative (one sided) confidence interval as discussed by Rumke (69), Louis (70), Hanley et al. (71) and Jovanovic & Levy (72) and known as the rule of three can be used if the Chi-square goodness of fit test (with a continuity correction) (73) cannot be applied.

**RESULTS**

Rheumatoid arthritis is an inflammatory progressive disease with more or less a very poor prognosis. In this context, the studies (74-99) considered for a re-analysis should help us to get a better understanding of this disease.

**Without EBV IgG antibody positivity no rheumatoid arthritis**

EBV VCA IgG antibodies can be used to investigate the relationship between EBV and RA.

**Claims**

**Null hypothesis: (no causal relationship)**

The presence of EBV VCA IgG antibodies is a necessary condition (a conditio sine qua non) of rheumatoid arthritis. In other words, the sample distribution agrees with the hypothetical (theoretical) distribution of a necessary condition.

**Alternative hypothesis: (causal relationship)**

The presence of EBV VCA IgG antibodies is not a necessary condition (a conditio sine qua non) of rheumatoid arthritis. In other words, the sample distribution does not agree with the hypothetical (theoretical) distribution of a necessary condition. The significance level (Alpha) below which the null hypothesis will be rejected is alpha=0.05.

**Proof**

The data reviewed by this article which investigated the relationship between EBV VCA IgG antibodies and rheumatoid arthritis are presented by Table 2. In total, 9 studies with 2507 cases and controls provided non self-contradictory data and were meta-analysed while the level of significance was alpha = 0.05. In particular, all studies provided significant evidence of a conditio sine qua non relationship between EBV VCA IgG antibodies and rheumatoid arthritis (\(X^2(\text{Calculated [conditio sine qua non]}) = 0.8597\) and is less than \(X^2(\text{Critical [conditio sine})

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The data reviewed by this article which investigated the relationship between EBV VCA IgG antibodies and rheumatoid arthritis are presented by Table 2. In total, 9 studies with 2507 cases and controls provided non self-contradictory data and were meta-analysed while the level of significance was alpha = 0.05. In particular, all studies provided significant evidence of a conditio sine qua non relationship between EBV VCA IgG antibodies and rheumatoid arthritis (\(X^2(\text{Calculated [conditio sine qua non]}) = 0.8597\) and is less than \(X^2(\text{Critical [conditio sine})
qua non]) = 16.919). In fact, the presence of EBV VCA IgG antibodies is a necessary condition (a condition sine qua non) of rheumatoid arthritis. Ultimately, for this reason, without the presence of EBV VCA IgG antibodies no rheumatoid arthritis.

Q. e. d.

**Without EBV EBNA IgG antibody positivity no rheumatoid arthritis**

**Claims**

**Null hypothesis**

The presence of EBV EBNA IgG antibodies is a necessary condition (a condition sine qua non) of rheumatoid arthritis. In other words, the sample distribution agrees with the hypothetical (theoretical) distribution of a necessary condition.

**Alternative hypothesis**

The presence of EBV EBNA IgG antibodies is not a necessary condition (a condition sine qua non) of rheumatoid arthritis. In other words, the sample distribution does not agree with the hypothetical (theoretical) distribution of a necessary condition.

The significance level (Alpha) below which the null hypothesis will be rejected is alpha = 0.05.

**Proof**

The data reviewed by this article which investigated the relationship between EBV EBNA IgG antibodies and rheumatoid arthritis are shown in Table 3. At this point it might be important that 7 studies with 794 cases and controls provided non self-contradictory data and were considered for a meta-analysis while the level of significance was alpha = 0.05. We can point to the fact that all 7 studies (Table 4) provided significant evidence of a condition sine qua non relationship between EBV EBNA IgG antibodies and rheumatoid arthritis ($X^2$ (Calculated [condition sine qua non]) = 3.1435 and is less than $X^2$ (Critical [condition sine qua non]) = 14.0671). By that very fact, the presence of EBV EBNA IgG antibodies is a necessary condition (a condition sine qua non) of rheumatoid arthritis. The last point suggests that without the presence of EBV EBNA IgG antibodies no rheumatoid arthritis.

Q. e. d.

**EBV is a cause of rheumatoid arthritis**

(The Study of Saal et al. (Table 10))

The presence of EBV DNA in synovial tissues is a possible method to show an etiological link between EBV and the pathogenesis of rheumatoid arthritis. Several studies published convincing results on this topic.

**Claims**

**Null hypothesis: (no causal relationship)**

There is no significant causal relationship between an infection by Epstein-Barr virus and rheumatoid arthritis. (k=0).

**Alternative hypothesis: (causal relationship)**

There is a significant causal relationship between an infection by Epstein-Barr virus and rheumatoid arthritis. (k¹0).

**Conditions.** Alpha level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for alpha level 5% is 3.841458821.

**Proof**

The data for this hypothesis test were provided by Saal et al. (Table 10) and are illustrated by the Table 10. The causal relationship k(Epstein-Barr virus, rheumatoid arthritis) was calculated as k = +0.2954 (p value (k) = 9.29228E-05; 95% CI (k) = [0.1213;0.4695]) while the level of significance was alpha = 0.05. The data of Saal et al. (Table 10) provide evidence that EBV is a sufficient condition ($X^2$(IMP) = 1.5203; $X^2$Critical(IMP) = 3.841458821) of rheumatoid arthritis while the cause effect relationship between EBV and RA is highly significant (k = +0.2954 (p value (k) = 9.29228E-05).

Q. e. d.

**EBV is a cause of rheumatoid arthritis**

(The Study of Takeda et al. (Table 11))

**Claims**

**Null hypothesis: (no causal relationship)**

There is no significant causal relationship between an infection by Epstein-Barr virus and rheumatoid arthritis. (k=0).

**Alternative hypothesis: (causal relationship)**

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**Proof**

The data for this hypothesis test were provided by Study of Takeda et al. (Table 11) and are illustrat-
ed by the Table 11. The causal relationship $k(\text{Epstein-Barr virus, rheumatoid arthritis})$ was calculated as $k = +0.5470$ ($p$ value ($k$) = 6.07959E-06; 95% CI ($k$) = [0.2630; 0.8310]) while the level of significance was $\alpha=0.05$. The data of Takeda et al. (Table 11) provide evidence that EBV is a sufficient condition ($X^2(\text{IMP}) = 0.0167$; $X^2$ Critical (IMP) = 3.841458821) of rheumatoid arthritis while the cause effect relationship between EBV and RA is highly significant ($k = +0.5470$ ($p$ value ($k$) = 6.07959E-06).

Q. e. d.

**EBV is the cause of rheumatoid arthritis**

The Study of Chiu et al. (Table 12)

**Claims**

**Null hypothesis:** (no causal relationship)

There is no significant causal relationship between an infection by Epstein-Barr virus and rheumatoid arthritis. ($k=0$).

**Alternative hypothesis:** (causal relationship)

There is a significant causal relationship between an infection by Epstein-Barr virus and rheumatoid arthritis. ($k \neq 0$).

**Conditions.** $\alpha$ level = 5%.

The two tailed critical Chi square value (degrees of freedom = 1) for $\alpha$ level 5% is 3.841458821.

**Proof**

The data for this hypothesis test were provided by Study of Chiu et al. (Table 12) and are illustrated by the Table 12. The causal relationship $k(\text{Epstein-Barr virus, rheumatoid arthritis})$ was calculated as $k = +1.0$ ($p$ value ($k$) = 4.32753E-10) while the level of significance was $\alpha=0.05$. The data of Study of Chiu et al. (Table 12) provide evidence that EBV is a necessary ($X^2(\text{SINE}) = 0.0109$; $X^2$ Critical (SINE) = 3.841458821), a sufficient ($X^2(\text{IMP}) = 0.0109$; $X^2$ Critical (IMP) = 3.841458821) and equally a necessary and sufficient condition ($X^2(\text{SINE and IMP}) = 0.0217$; $X^2$ Critical (SINE and IMP) = 3.841458821) of rheumatoid arthritis while the cause effect relationship is highly significant ($k = +1.0$; $p$ value ($k$) = 4.32753E-10). Epstein-Barr virus is the cause of rheumatoid arthritis ($k = +1.0$; $p$ value ($k$) = 4.32753E-10).

Q. e. d.

**DISCUSSION**

Epstein-Barr Virus discovered 1964 by Epstein et al. (100) is a widely disseminated lymphotropic herpes virus. As key results, several studies suspected that particularly Epstein-Barr virus is involved in etiology of rheumatoid arthritis. Catalano et al. (21) reported that patients with RA had a significantly higher frequency and titer of rheumatoid arthritis-associated nuclear antigen (anti-RANA) antibodies than did control subjects and confirmed the previous results of Alspaugh and Tan (18). Using the protein blot technique, Billings et al. (23) were able to provide evidence that rheumatoid arthritis nuclear antigen (RANA) and Epstein-Barr virus nuclear antigen identify the same polypeptide.

However, data about EBV burden in RA patients reported have been contradictory and the role of EBV still remains elusive. Indeed, on this matter, as with so many other major medical issues, several reviews101, 102 and meta-analysis were not able to find a definite solution on this fundamental topic. Thus far, it is not excluded that this meta-analyses is susceptible to different kind of publication bias. In its broadest sense, the studies analysed differ in various aspect. Thus, the question arises why not all patients were diagnosed according to the American Rheumatism Association 1987 revised criteria for the classification 103 of RA. While some studies considered for a meta-analysis provided no diagnostic criteria for the diagnosis of rheumatoid arthritis other studies utilised a form of the American College of Rheumatology (ACR) or American Rheumatology Association criteria. Additionally, reporting of data of some studies are to some extent unsatisfactory, because not all studies provided detailed cut-off values for EBV sero-positivity. RA patients and non-RA controls both were tested quantitatively for different antibodies against Epstein-Barr virus while using different substrates or kits or antigens and various technologies. Hence we need to take into consideration under what conditions is it appropriate to use antibodies against Epstein-Barr virus to investigate the relationship between EBV and rheumatoid arthritis? To date it is known that IgG molecules with two antigen binding sites are created and released by human plasma B cells not without any reason but i. e. to control an infection in human body. Especially IgM, IgG et cetera molecules are not existing for ever but suffer a kind of pharmacokinetics. The half-life (104) for total IgG was found to be
25.8 days. In this context EBV antibodies are major components of human humoral immunity allowing controlling an EBV infection of human body tissues through several mechanisms. A natural concern is whether EBV antibodies suffer a turnover rate with regard to the infectious status. Several factors can influence the pharmacokinetics of EBV antibodies. The half-lives for antibodies specific for Epstein-Barr virus antigens depend on EBV infection status. In the case of recent EBV infection or during the course of EBV reactivation the humoral response of human immune system against EBV antigens will lead to different changes in antibodies specific for Epstein-Barr virus antigens. An acute EBV (re-) infection is indicated by the presence of VCA IgM and VCA IgG but without EBNA-1 IgG. Typical for a past EBV infection is the presence of VCA IgG and EBNA-1 IgG but without VCA IgM (105).

At the very least, enough is published to convince our self that after a primary EBV infection, EBV persists for life in vivo in a quiescent state in resting human memory B cells (106) which circulate in the peripheral blood. This fact considerably leads to the conclusion that VCA IgG or EBNA IgG provide evidence of an EBV infection of human body and are therefore helpful in causal analysis. And yet, despite contradictory results several studies give convincing evidence of the linkage between EBV and RA. Many studies demonstrated remarkable higher levels of different serum antibodies against Epstein-Barr virus in patients suffering from rheumatoid arthritis than in healthy controls (21, 22, 24, 76, 107, 108, 109). Baecklund et al. (110) provided evidence that a high inflammatory activity of RA rather than the treatment of RA is a major risk determinant of lymphoma in a subset of patients with RA.

Sherina et al. (99) conducted the largest epidemiological study to date and investigated the prevalence of EBV, human cytomegalovirus (CMV) and parvovirus B19 antibodies by ELISA in serum samples from 990 RA patients and 700 controls. The prevalence of EBV IgG was 98.3% in patients with RA and 97.0% in controls. Parvovirus B19 IgG were detected in 75.8% of patients with RA and in 72.8% of healthy controls. CMV IgG was documented in 75.9% of controls and in 72.2% of patients with RA. For the first time, the viruses EBV, CMV and parvovirus B19 have been examined by Sherina et al. (99) in the context of a very large and impressive epidemiological study in patients with RA and in non-RA subjects. Sherina et al. used the presence of anti-viral antibodies as surrogate markers for viral infection.

The data of Sherina et al. (99) with a sample size of n= 1690 cases and controls concerning the relationship between parvovirus B19 and rheumatoid arthritis (Table 7) were not self-contradictory and could be used for further analysis. The data of Sherina et al. (99) do not support the Null-hypothesis: without parvovirus B19 infection no rheumatoid arthritis (X² (SINE) Calculated = 57.9396 and thus far greater than X² (SINE) Critical = 3.841458821). The data of Sherina et al. (99) do not support the Null-hypothesis: if parvovirus B19 infection then rheumatoid arthritis (X² (IMP) Calculated = 205.3791 and thus far greater than X² (IMP) Critical = 3.841458821). In other words, according to the data of Sherina et al. (99) parvovirus B19 is neither a cause nor the cause of rheumatoid arthritis (Table 7) even if statistically not independent (111) of each other.

Contradicting the study Sherina et al. (99), Takahashi (112) et al., 1998 found Human parvovirus B19 DNA (B19) in the synovium of 30/39 RA patients in contrast to 9/57 controls while neither the study of Kerr (113) et al. nor the study of Naciute (114) et al. with B19 DNA in 30/118 of RA patients vs. 9/49 in healthy controls confirmed the data of Takahashi (112) et al., 1998.

The data of Sherina et al. (99) concerning the relationship between CMV and rheumatoid arthritis were not self-contradictory (Table 8) and could be considered for further analysis. The data of Sherina et al. (99) do not support the Null-hypothesis: without CMV infection no rheumatoid arthritis (p(SINE) = 0.8376; X²(SINE) Calculated = 75.7875 and thus far greater than X² (SINE) Critical = 3.841458821). The data of Sherina et al. (99) do not support the Null-hypothesis: if CMV infection then rheumatoid arthritis (p(IMP)=0.6852; X²(IMP) Calculated =226.2301 and thus far greater than X² (IMP) Critical = 3.841458821). Thus far, according to the data of Sherina et al. (99) it appears not to be highly probable that CMV might somehow be involved in the pathogenesis of RA. CMV is neither a cause nor the cause (Table 8) of RA (k=-0.0405; p value (k) =0.2750 and is thus far not greater than X² (SINE) Critical = 3.841458821, romanian journal of rheumatology – volume xxvii, no. 4, 2018

null hypothesis:
k > 0; p value (k) = 0.029020429). This Null-hypothesis is supported by other studies too. In other words, according to the data (Table 9) of the very large epidemiological study conducted by Sherina et al. (99) EBV infection is the cause of rheumatoid arthritis.

However, even EBV DNA analysis provided view contradictory results; while some studies failed to detect EBV DNA in RA patients (115) other studies were successful. Saal et al. (88) (Table 10) investigated the presence of the Epstein-Barr virus (EBV) in rheumatoid arthritis (RA) synovium and concluded that EBV is an environmental risk factor for RA. According to the study of Saal et al. (88) there is a highly significant cause effect relationship (Table 10) between an EBV infection of human joints and RA (k =+0.2954; p value (k) =9.29228E-05) while the conditio per quam relationship between EBV and RA is significant. In other words, if EBV infection of human joints then RA (p(IMP)=0.9515; X² (IMP)=1.5203).

Takeda et al. (91) (Table 11) detected the existence of EBV DNA by PCR in the synovial tissue in 15 of the 32 samples from the RA patients (47%), but not in any of the 30 osteoarthritis patients (Table 11). Takeda et al. (91) were able to provide evidence that an infection of human joints by EBV is a conditio per quam of rheumatoid arthritis. In other words, according to the study of Takeda et al. (91) (Table 11) if infection of human joints by EBV then RA (p (IMP)=0.9515; X² (IMP)=1.5203).

Using real-time polymerase chain reaction Balandraud et al. (116) were able to document that Epstein-Barr virus DNA load in the peripheral blood (116) of patients with rheumatoid arthritis was increased almost 10-fold.

In situ hybridization

In-situ hybridization (ISH), has been described in the year 1969 by Joseph G. Gall (117). According to Fan & Gulley (118), In situ hybridization (ISH) to Epstein-Barr virus (EBV)-encoded small RNA1 (EBER1) by ISH in the synovial tissues taken from 23 patients with rheumatoid arthritis and 13 patients with OA. The RA patients were diagnosed according to the American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis (103). All synovial samples from RA showed positive expression of EBER1 (23/23,100%), but none of the control group patients (0/13).

According to the study of Chiu et al. (96) (Table 12), an EBV virus infection is a necessary condition (p (SINE) =1; X²(SINE ) =0.0109), an EBV virus infection is a sufficient condition (p (IMP)=1; X² (IMP)=0.0109) and an EBV virus infection is a necessary and sufficient condition (p(SINE AND IMP) = 1; X²(SINE AND IMP) = 0.0217) of rheumatoid arthritis while the cause effect relationship (Table 12) between an EBV infection and RA is highly significant (k = +1; p value (k) = 4.32753E-10).

Mehraein et al. (119) investigated the influence of synovial virus infections in rheumatoid arthritis, and found evidence of increased synovial persistence of EBV in 5/29 rheumatoid arthritis (RA) patients.

Mahabadi et al. (98) investigated Epstein-Barr virus DNA by PCR in synovial fluid of 50 rheumatoid arthritis patients and detected EBV DNA by PCR in 30 cases (60%). Mahabadi et al. (98) concluded that EBV may play a role in the pathogenesis of RA. A control group was not provided and it was not possible to consider the data for causal analysis.

Although it has been investigated and speculated for over 40 years that Epstein-Barr virus is a strong candidate to contribute to the cause of RA definite evidence was wanting. Considering the half-life (120) of EBV antibodies and the results of the reviews (121) mentioned, the studies re-analysed in the present article indicate a high degree of confidence that an EBV infection is the cause of RA and the etiology of rheumatoid arthritis no longer remains unknown. The lack of appropriate ancient medical texts regarding rheumatoid arthritis has forced many researchers to acknowledge the first description of RA by modern medicine to Augustin Jacob Landré-Beauvais (122, 123) from the year 1800 published in his dissertation. In the year 2018 and about 218 years later, the cause of rheumatoid arthritis is finally identified.
CONCLUSION

The results of the present study are consistent with the hypothesis that there is a relationship between EBV and RA and give further evidence of the linkage between EBV and RA. The data not only do support the hypothesis that EBV infection is some-

how involved in the pathogenesis of RA but demand us to accept that EBV is the cause of RA (k = +1.0000; p value (k) = 4.32753E-10).

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