Human Cytomegalovirus: The Cause Of Glioblastoma Multiforme

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Abstract

Objective: The relationship between Human cytomegalovirus (HCMV) and glioblastoma multiforme (GBM) is investigated.

Methods: A systematic review and re-analysis of some impressive key studies was conducted aimed to answer the following question. Is there a cause-effect relationship between HCMV and GBM? The method of the condition sine qua non relationship was used to proof the hypothesis whether the presence of HCMV guarantees the presence of GBM. In other words, without HCMV no GBM. The mathematical formula of the causal relationship k was used to proof the hypothesis, whether there is a cause-effect relationship between HCMV and GBM. Significance was indicated by a p-value of less than 0.05.

Results: The studies analysed were able to provide strict evidence that HCMV is a necessary condition (a condition sine qua non) of GBM. Furthermore, the cause-effect relationship between HCMV and GBM (k = +1, p value < 0.0001) was highly significant.

Conclusion: Without a human cytomegalovirus infection no glioblastoma multiforme. Human cytomegalovirus is the cause of glioblastoma multiforme.

Keywords: Cytomegalovirus, Glioblastoma multiforme,
Causal relationship

1. Introduction

Glioblastoma multiforme (GBM), affecting children and adults and equivalent to a grade IV diffuse astrocytoma, is a highly lethal brain (Zhu et al., 2002) tumour to such an extent that to date the majority of affected patients are dying from their disease by 2.5 years following diagnosis (Smoll et al., 2013). Glioblastoma multiforme consist primarily of neoplastic astrocytes but includes also other non-neoplastic cell types (Yuan et al., 2004) like neural stem cells, macrophages et cetera. In point of fact, even if Glioblastoma multiforme progresses rapidly and is fatal within a very short time despite current therapies, to date the aetiology of glioblastoma multiforme is completely unknown. Among several risk factors supposed to be involved in glioblastoma including exposure to electrical or magnetic fields or ionizing radiation (Ohgaki, 2009) cytomegalovirus too has been proposed as a contributing agent of glioblastoma multiforme. Human cytomegalovirus (HCMV) is a beta herpes virus which more or less asymptotically (Priel et al., 2015) infects 50% to 90% of the adult (Yi et al., 2013; Najafi et al., 2016) human population. Even if a foetus itself is not protected against a vertical transmission. During maternal primary infection, and to a lesser extent during recurrent infection, human cytomegalovirus can translocate the placental barrier and can cause infection of the developing foetus too. Human cytomegalovirus is occurring in 0.5–2% of pregnancies in Europe and the United States (Kenneson et al., 2007; Wang et al., 2011) and neonatal infections (Fowler et al., 2018) caused by human cytomegalovirus have been reported too. In particular, many breast milk-acquired infections in premature infants are asymptomatic. Several studies demonstrated the presence of human cytomegalovirus in glioblastoma tissues suggesting that HCMV may participate in tumour pathogenesis. But the relationship between GBM and HCMV is controversial because many other studies were unable to detect HCMV in Glioblastoma multiforme tissues.
2. Material and methods

2.1. Material

2.1.1. Search strategy
To answer the questions addressed in this paper, the electronic database PubMed was searched for appropriate studies conducted in any country which investigated the relationship between human cytomegalovirus and glioblastoma multiforme i.e. sero-epidemiologically or by polymerase chain reaction (PCR) et cetera. The search in Pubmed was performed while using some medical key words like "case control study" and "cytomegalovirus" and "glioblastoma multiforme" et cetera. The articles found where saved as a *.txt file while using the support of Pubmed (Menu: Send to, Choose Radio Button: File, Choose Format: Abstract (text). Click button "create file"). The created *.txt file was converted into a *.pdf file. The abstracts where studied within the *.pdf file. Those articles were considered for a re-view which provided access to data without any data access barrier. Additionally the reference list of identified articles was used as a potential source of articles appropriate for this study.

2.1.2. The 2x2 table
The meaning of the abbreviations $a$, $b$, $c$, $d$, $N_i$ of the data table used are explained by a 2 by 2-table Table 1.

<table>
<thead>
<tr>
<th>Condition A, (risk factor)</th>
<th>Conditioned B, (Outcome)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes = +1</td>
<td>Yes = +1</td>
</tr>
<tr>
<td>$a_i$</td>
<td>$b_i$</td>
</tr>
<tr>
<td>Total</td>
<td>$B_i$</td>
</tr>
</tbody>
</table>

In general it is $(a+c+b+d) = A_i$, $(c+d) = B_i$, $(a+b+c+d) = N_i$. Equally, it is $B_i + B_i = A_i + A_i = N_i$. In this context, it is $p(a_i) = p(A_i \cap B_i)$, $p(a_i) = p(a_i) + p(b_i)$ or $p(A_i) = p(A_i \cap B_i)$ + $p(b_i) = p(A_i \cap B_i) + p(A_i \cap B_i)$ while $p(a_i)$ is not defined as $p(a_i)$. In the same context, it is $p(B_i) = p(a_i) + p(c_i) = p(A_i \cap B_i) + p(c_i)$ and equally that $p(B_i) = 1 - p(B_i) = p(b_i) + p(d_i)$. Furthermore, the joint probability of $A_i$ and $B_i$ is denoted by $p(A_i \cap B_i)$. Thus far, it is $p(A_i \cap B_i) = p(A_i) - p(b_i) = p(A_i) - p(c_i)$ or in other words it follows that $p(B_i) + p(b_i) - p(c_i) = p(A_i)$. Thus far, define $\Lambda = p(b_i) - p(c_i)$, Einstein’s term $\Lambda$ under conditions of probability theory and we obtain $p(B_i) + \Lambda = p(A_i)$. In general, it is $p(a_i) + p(c_i) + p(b_i) + p(d_i) = 1$.

2.1.3. The studies analyzed
The studies analyzed are viewed by the Table 2.

Table 2. Without human cytomegalovirus infection no glioblastoma multiforme.

<table>
<thead>
<tr>
<th>Study Id</th>
<th>Country</th>
<th>N</th>
<th>Case Positive</th>
<th>Case Total</th>
<th>Control positive</th>
<th>Control Total</th>
<th>X²(Sine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rahbar et al. 2013</td>
<td>SE</td>
<td>80</td>
<td>79</td>
<td>80</td>
<td>-</td>
<td>-</td>
<td>0,003125</td>
</tr>
<tr>
<td>Rahbar et al., 2013</td>
<td>SE</td>
<td>75</td>
<td>74</td>
<td>75</td>
<td>-</td>
<td>-</td>
<td>0,00333333</td>
</tr>
<tr>
<td>Cobbs et al., 2002</td>
<td>USA</td>
<td>45</td>
<td>22</td>
<td>22</td>
<td>0</td>
<td>23</td>
<td>0,011363636</td>
</tr>
<tr>
<td>Scheuer et al., 2008</td>
<td>USA</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td>0,011904762</td>
</tr>
<tr>
<td>Slinger E, et al. 2010</td>
<td>NL</td>
<td>21</td>
<td>20</td>
<td>21</td>
<td>-</td>
<td>-</td>
<td>0,011904762</td>
</tr>
<tr>
<td>Straat K, et al. 2009</td>
<td>USA</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td>0,025</td>
</tr>
<tr>
<td>Dzurzynski et al., 2011</td>
<td>USA</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>-</td>
<td>-</td>
<td>0,05</td>
</tr>
<tr>
<td>Mitchell et al., 2008</td>
<td>USA</td>
<td>45</td>
<td>42</td>
<td>45</td>
<td>-</td>
<td>-</td>
<td>0,138888889</td>
</tr>
<tr>
<td>Holdhoff et al., 2017</td>
<td>USA</td>
<td>15</td>
<td>8</td>
<td>15</td>
<td>-</td>
<td>-</td>
<td>2,816666667</td>
</tr>
</tbody>
</table>
2.1.3. The study of Cobbs et al.

Cobbs et al. (Cobbs et al., 2002) performed immunohistochemistry investigations (IHC) on paraffin sections from 22 malignant glioma surgical specimens obtained from non-immunocompromised patients to determine whether HCMV was present in malignant glioma. Cobbs et al. detected IE1–72 immunoreactivity in 27 of 27 malignant glioma biopsy specimens but in none of the 23 controls. The data as obtained by Cobbs et al. are view by the Table 3.

Table 3. The Study of Cobbs et al., 2002

<table>
<thead>
<tr>
<th>HCMV infection</th>
<th>Glioblastoma multiforme (GBM)</th>
<th>&lt;B&gt;</th>
<th>&lt;A&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>Yes</td>
<td>22</td>
<td>0</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22</td>
<td>23</td>
</tr>
</tbody>
</table>

k = 1
p value (k) = 1.9703E-11

Without <A> NO <B>.

\[ p(\text{SINE}) = 1 \]

\[ X^2(\text{SINE}) = 0.01136364 \]

IF <A> THEN <B>

\[ p(\text{IMP}) = 1 \]

\[ X^2(\text{IMP}) = 0.01136364 \]

\[ p(\text{SINE} \land \text{IMP}) = 1 \]

\[ X^2(\text{SINE} \land \text{IMP}) = 0.02272727 \]

2.1.4. The control group of infants

Several of the studies presented are missing an own control group. Even if this has no influence on the correct calculation of the chi square value of the data, it has influence on the validity of the causal relationship. In order to be able to perform causal relationship calculations, it is possible to construct a theoretical or fictive control group of infants. In this context, Fowler et al. (Fowler et al., 2018) evaluated from 2007 to 2012 the prevalence of a congenital cytomegalovirus infection (HCMV) in a cohort of 100332 infants screened for HCMV while in the hospital. The overall prevalence of HCMV in the cohort of infants investigated was in the range between 1.0 per 1000 live births and 9.5 per 1000 live births. In other words, a cytomegalovirus infection can be found in less than 1% of infants while glioblastoma multiforme cases in infants have not been reported so far which is the reason to use infants as a control group. The data of the study of Fowler et al. (Fowler et al., 2018) are of use while constructing a factitive control group of infants. Such a factitive control group of infants can be used as a substitute for the missed control group in the studies presented.
2.2. Methods

2.2.1. Statistical analysis
All statistical analyses (Barukčić, 1989; Barukčić, 2017a; Barukčić, 2017b; Barukčić, 2017c; Barukčić, 2018a; Barukčić, 2018b; Barukčić, 2018c) were performed with Microsoft Excel version 14.0.7166.5000 (32-Bit) Software (Microsoft GmbH, Munich, Germany). The level of significance was set to 0.05. The probabilities of the contingency table are viewed by the following table (Table 4).

Table 4. The probabilities of a contingency table

<table>
<thead>
<tr>
<th>Condition A&lt;sub&gt;t&lt;/sub&gt;</th>
<th>Yes = +1</th>
<th>No = +0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes = +1</td>
<td>p(α&lt;sub&gt;t&lt;/sub&gt;) = p(A&lt;sub&gt;t&lt;/sub&gt; ∩ B&lt;sub&gt;t&lt;/sub&gt;)</td>
<td>p(b&lt;sub&gt;t&lt;/sub&gt;)</td>
<td>p(A&lt;sub&gt;t&lt;/sub&gt;)</td>
</tr>
<tr>
<td>No = +0</td>
<td>p(c&lt;sub&gt;t&lt;/sub&gt;)</td>
<td>p(d&lt;sub&gt;t&lt;/sub&gt;)</td>
<td>p(Δ&lt;sub&gt;t&lt;/sub&gt;)</td>
</tr>
<tr>
<td>Total</td>
<td>p(B&lt;sub&gt;t&lt;/sub&gt;)</td>
<td>p(B&lt;sub&gt;t&lt;/sub&gt;)</td>
<td>1</td>
</tr>
</tbody>
</table>

2.2.2. Independence
In the case of independence of A<sub>t</sub> and B<sub>t</sub> it is generally valid that

\[ p(A_t \cap B_t) = p(A_t) \times p(B_t) \] (1)

2.2.3. Exclusion (A<sub>t</sub> excludes B<sub>t</sub> and vice versa relationship)
The mathematical formula of the exclusion relationship (A<sub>t</sub> excludes B<sub>t</sub> and vice versa) of a population was defined as

\[ p(A_t | B_t) = \frac{b_t + c_t + d_t}{N_t} = 1 - p(a_t) = p(b_t) + p(c_t) + p(d_t) = p(c_t) + (1 - p(B_t)) = p(b_t) + (1 - p(A_t)) = +1 \] (2)

and used to proof the hypothesis: A<sub>t</sub> excludes B<sub>t</sub> and vice versa.

2.2.4. Necessary condition (conditio sine qua non)
The mathematical formula of the necessary condition relationship (conditio sine quam non) of a population was defined as

\[ p(A_t \leftarrow B_t) = \frac{a_t + b_t + d_t}{N_t} = p(a_t) + p(b_t) + p(d_t) = p(a_t) + (1 - p(B_t)) = +1 \] (3)

and used to proof the hypothesis: without A<sub>t</sub> no B<sub>t</sub>.

2.2.5. Sufficient condition (conditio per quam)
The mathematical formula of the sufficient condition relationship (conditio per quam) of a population was defined as

\[ p(A_t \rightarrow B_t) = \frac{a_t + c_t + d_t}{N_t} = p(a_t) + p(c_t) + p(d_t) = p(d_t) + p(B_t) = +1 \] (4)

and used to proof the hypothesis: if A<sub>t</sub> then B<sub>t</sub>. 

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2.2.6. The $X^2$ goodness of fit test of a necessary condition

Under conditions where the chi-square goodness of fit test (Pearson, 1900) cannot be used it is possible to use an approximate and conservative (one sided) confidence interval known as the *rule of three* (Rumke, 1975; Hanley et al. 1983; Louis, 1981; Jovanovic et al., 1997). According to the definition of the conditio sine qua non relationship it is

$$p(A_t \cap B_t) + (1 - p(B_t)) \equiv +1$$  \tag{5}

or

$$p(A_t \cap B_t) + 1 - p(B_t) \equiv +1$$  \tag{6}

Rearranging this equation, we obtain the essential foundation of the conditio sine qua non relationship as

$$p(A_t \cap B_t) = p(B_t)$$  \tag{7}

and equally our starting point of the derivation of chi-square value of the conditio sine qua non relationship. Multiplying equation before by the population or sample/population size $N$, it is

$$N \times p(A_t \cap B_t) \equiv N \times p(B_t)$$  \tag{8}

or

$$N \times p(A_t \cap B_t) - N \times p(B_t) = 0$$  \tag{9}

The square operation yields

$$\left( N \times p(A_t \cap B_t) - N \times p(B_t) \right) \times \left( N \times p(A_t \cap B_t) - N \times p(B_t) \right) = 0 \times 0$$  \tag{10}

Dividing by $N\times p(B_t)$ we obtain

$$\frac{\left( N \times p(A_t \cap B_t) - N \times p(B_t) \right)^2}{N \times p(B_t)} = 0$$  \tag{11}

which is equivalent with

$$\frac{(a_t - (B_t))^2}{(B_t)} = \frac{(a_t - (a_t + c_t))^2}{(B_t)} = \frac{(c_t)^2}{(B_t)} = 0$$  \tag{12}

Adding $((b_t+d_t)-(B_t))^2/B_t = ((b_t+d_t)-(b_t+d_t))^2/B_t = 0$ yields

$$\frac{(c_t)^2}{(B_t)} + 0 = 0 + 0$$  \tag{13}

Using the *continuity correction*, the chi-square value of a conditio sine qua non distribution before changes to

$$\chi^2 (SINE) \equiv \frac{(c_t - \frac{1}{2})^2}{(B_t)} + 0 = 0$$  \tag{14}

The use of the continuity correction should follow the rules of statistics as established and valid today. This definition of the $X^2$ distribution of a *conditio sine qua non* distribution (degrees of freedom = 2-1=1) is more precise than already published formulas. In this context, it is not necessary to improve the definition of the $X^2$ distribution of a *conditio per quam* distribution as already published. A statistically significant conditio sine qua non relationship demands a causal relationship $k$ which is $k > 0$, otherwise the result of a study should be treated with cautious.
2.2.7. The $X^2$ goodness of fit test of the exclusion relationship

According to the definition of the exclusion relationship it is

$$p(b_i) + p(c_i) + p(d_i) \equiv +1$$

Rearranging this equation, we obtain

$$p(b_i) = 1 - p(c_i) - p(d_i) = 1 - (p(c_i) + p(d_i)) \equiv 1 - p(A_i) = p(A_i)$$

and

$$p(c_i) = 1 - p(b_i) - p(d_i) \equiv 1 - (p(b_i) + p(d_i)) = 1 - p(B_i) = p(B_i)$$

The chi square goodness of fit test of the exclusion relationship can be derived as follows.

$$\chi^2(b_i) \equiv \frac{(N \times p(b_i) - N \times p(A_i))^2}{N \times p(A_i)} = \frac{(b_i - (a_i + b_i))^2}{A_i} = \frac{(-a_i)^2}{A_i} = 0$$

and as

$$\chi^2(c_i) \equiv \frac{(N \times p(c_i) - N \times p(B_i))^2}{N \times p(B_i)} = \frac{(c_i - (a_i + c_i))^2}{B_i} = \frac{(-a_i)^2}{B_i} = 0$$
The chi square value with degree of freedom 2-1=1 of the exclusion relationship with a continuity correction can be calculated as

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

(20)

A statistically significant exclusion relationship demands a causal relationship $k$ which is $k < 0$ otherwise the results of a study should be interpreted with some cautious.

2.3.5. The mathematical formula of the causal relationship $k$

The mathematical formula of the causal relationship $k$ (Barukčić, 1989; Barukčić, 2017a; Barukčić, 2017b; Barukčić, 2017c; Barukčić, 2018a; Barukčić, 2018b; Barukčić, 2018c) is defined at every single event, at every single Bernoulli trial $t$, as

$$k(A_t, B_t) = \frac{(p(A_t \cap B_t) - (p(A_t) \times p(B_t)))}{\sqrt{(p(A_t) \times p(A_t)) \times (p(B_t) \times p(B_t))}}$$

(21)

where $A_t$ denotes the cause and $B_t$ denotes the effect. The chi-square distribution can be applied to determine the significance of causal relationship $k$. Correlation (Pearson, 1896) is not causation, causation is not correlation. The relationship between correlation and causation is discussed already in many publications. This does not necessarily imply that repeating itself over and over again may contribute anything new to further scientific progress.

2.3.6. The chi square distribution

The following critical values of the chi square distribution as visualized by Table 5 are used in this publication.

Table 5. The critical values of the chi square distribution (degrees of freedom: 1).

<table>
<thead>
<tr>
<th>p-Value</th>
<th>One sided $X^2$</th>
<th>Two sided $X^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0,1000000000</td>
<td>1,642374415</td>
<td>2,705543454</td>
</tr>
<tr>
<td><strong>0,0500000000</strong></td>
<td><strong>2,705543454</strong></td>
<td><strong>3,841458821</strong></td>
</tr>
<tr>
<td>0,0400000000</td>
<td>3,06490172</td>
<td>4,217884588</td>
</tr>
<tr>
<td>0,0300000000</td>
<td>3,537384596</td>
<td>4,709292247</td>
</tr>
<tr>
<td>0,0200000000</td>
<td>4,217884588</td>
<td>5,411894431</td>
</tr>
<tr>
<td>0,0100000000</td>
<td>5,411894431</td>
<td>6,634896601</td>
</tr>
<tr>
<td>0,0010000000</td>
<td>9,549535706</td>
<td>10,82756617</td>
</tr>
<tr>
<td>0,0001000000</td>
<td>13,83108362</td>
<td>15,13670523</td>
</tr>
<tr>
<td>0,0000100000</td>
<td>18,18929348</td>
<td>19,51142096</td>
</tr>
<tr>
<td>0,0000010000</td>
<td>22,59504266</td>
<td>23,92812698</td>
</tr>
<tr>
<td>0,0000001000</td>
<td>27,03311129</td>
<td>28,37398736</td>
</tr>
<tr>
<td>0,0000000100</td>
<td>31,49455797</td>
<td>32,84125335</td>
</tr>
<tr>
<td>0,0000000010</td>
<td>35,97368894</td>
<td>37,32489311</td>
</tr>
<tr>
<td>0,0000000001</td>
<td>40,46665791</td>
<td>41,82145620</td>
</tr>
</tbody>
</table>

The chi square distribution
3. Results

3.1. Without a human cytomegalovirus infection no glioblastoma multiforme.

Claims.
Null hypothesis:
A cytomegalovirus infection is a necessary condition (a conditio sine qua non) of glioblastoma multiforme. In other words, the sample distribution of the study analyzed agrees with the hypothetical (theoretical) distribution of a necessary condition.

Alternative hypothesis:
A cytomegalovirus infection is not a necessary condition (a conditio sine qua non) of glioblastoma multiforme. In other words, the sample distribution of the study analyzed 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 results of the re-analyses of the data reviewed by this article (Table 2) which investigated the relationship between a cytomegalovirus infection and glioblastoma multiforme are viewed by the table (Table 2). Altogether, 9 studies were meta-analyzed while the level of significance was alpha = 0,05. In toto, 9 from 9 studies provide significant evidence of a conditio sine qua non relationship between a cytomegalovirus infection and glioblastoma multiforme. The most studies were performed without a control group which has no effect on the calculation of the chi square value. The study of Cobbs et al. provided evidence of a highly significant cause effect relationship (k = +1, p value = 1,9703E-11) between a cytomegalovirus infection and glioblastoma multiforme while the sample size was greater than 30. Consequently, it was possible to use the normal distribution. In other words, the data analyzed support the Null-hypothesis: without a cytomegalovirus infection no glioblastoma multiforme. Human cytomegalovirus is the cause of glioblastoma multiforme (k = +1, p value = 1,9703E-11). Q. e. d.

3.2. HCMV is the cause of glioblastoma multiforme.
The causal relationship between HCMV and GBM is difficult to proof due to fact of missing control groups in the studies presented. Because of this, some of the cases are compared with a fictive control group of infants (Table 6). The reason for such a procedure is that GMB has not been reported in infants so far which justifies such an undertaking. In contrast to this, a HCMV infection has been documented in infants. Human cytomegalovirus infects the adult human population (Yi et al., 2013; Najafi et al., 2016) but infants (Fowler et al., 2018) too. HCMV is able to translocate the placental barrier and is occurring in about 0,5–2% of pregnancies in Europe and the United States (Kenneson et al., 2007; Wang et al., 2011). In point of fact, Fowler et al. (Fowler et al., 2018) were able to document a cytomegalovirus infection in less than 1% of infants. This fact is the foundation for the construction of a fictive control group of infants for further use. In general, if we compare the GMB cases with a control group of fictive infants we must consider that not more than 1 % of infants can be HCMV positive, even if infants were not investigated again by a study analyzed.

Claims.
Null hypothesis:
A cytomegalovirus infection is not the cause of glioblastoma multiforme. In other words, k = 0.

Alternative hypothesis:
A cytomegalovirus infection is the cause of glioblastoma multiforme. In other words, k ≠ 0.

The significance level (Alpha) below which the null hypothesis will be rejected is alpha=0,05.

Proof.
The results of the re-analyses of the data reviewed by this article (Table 2) which investigated the causal relationship between a cytomegalovirus infection and glioblastoma multiforme are viewed by the table (Table 9). Altogether, 9 studies were meta-analyzed while the level of significance was alpha = 0,05. In toto, 9 from 9 studies provide significant evidence of a causal relationship between a cytomegalovirus infection and glioblastoma multiforme. In the same respect, a cytomegalovirus infection is a necessary, a sufficient and a necessary and sufficient condition of glioblastoma multiforme (Table 6). Even if the most cases were compared with a fictive control group of infants, the conclusion is inescapable. Human cytomegalovirus is the cause of glioblastoma multiforme. Q. e. d.
According to the study of Hung et al. (Hung et al., 2017) with a sample size of N = 6260, we accept the Null-hypothesis: coronary heart disease (CAD) and an etanercept therapy are excluding each other (X²(Excl) = 9,2166667, p value(k) = 0.0066667) with the consequence that etanercept is a highly effective antidot against CAD. Q.e.d.
Based on the data of Hung et al. (Hung et al. 2017), etanercept is highly effective against CAD. CAD itself is caused by HCMV (Barukčić, 2018d). Conclusion. Etanercept is highly effective against HCMV. Glioblastoma multiforme is caused by HCMV. Therefore, it is more than justified to considered etanercept for the therapy of glioblastoma multiforme.

Anti-tumor necrosis factor alpha (anti-TNF- alpha) antibodies are widely used to treat several inflammatory diseases and the treatment with a TNFalpha antagonist seems to be associated with various adverse (Bongartz et al., 2006) events. Petersen et al. (Petersen et al., 2008) reported the history of a 37-year-old male who developed a primary HCMV infection following a month of therapy with etanercept 50mg twice weekly. After discontinuation of etanercept, the patient recovered. Six months later, the same patient restarted on etanercept without HCMV reactivation. This increasingly highlights the importance to stress out the nature of coincidence of events. The coincidence of an etanercept therapy and a new HCMV infection does not proof a causal relationship between both. Justification: After discontinuation of the therapy with etanercept, the same patient restarted on etanercept without (Petersen et al., 2008) reactivating a HCMV infection. Still, due to Petersen’s case (Petersen et al., 2008) the question is justified, whether etanercept’s property to control a HCMV infection is dose dependent.

3.4. Leflunomide and glioblastoma multiforme

Leflunomide (N-(4’-trifluromethylphenyl)-5-methylisoxazole-4-carboxamide) introduced for the treatment of rheumatoid arthritis in 1998 is an inhibitor of protein kinase activity and pyrimidine synthesis. Leflunomide with its antiviral properties (Teschner et al., 2010) appears not to inhibit viral DNA synthesis but rather seems to interfere with virion assembly (Sudarsanam et al., 2006). Leflunomide as an inhibitor of protein kinase activity and pyrimidine synthesis is an immunosuppressive agent which is effective against CMV (Waldman et al., 1999; John et al., 2004; Sudarsanam et al., 2006; Verkaik et al., 2013; Lu et al., 2015). Cytomegalovirus (CMV) itself is a major cause of mortality especially among pharmacologically immunosuppressed transplant recipients. Furthermore, HCMV is the cause of CAD (Barukčić, 2018d). An anti-CMV drug like leflunomide should be able to decrease coronary artery disease events like acute myocardial infarction.

Claims.
Null hypothesis: Leflunomide and acute myocardial infarction are excluding each other.

Alternative hypothesis: Leflunomide and acute myocardial infarction are not excluding each other. The significance level (Alpha) below which the null hypothesis will be rejected is alpha=0,05.

Proof.

Suissa et al. (Suissa et al., 2006) investigated whether the risk of acute myocardial infarction (AMI) is associated with several disease-modifying antirheumatic drugs (DMARDs) commonly used in rheumatoid arthritis (RA) therapy including leflunomide. The data as obtained by Suissa et al. are viewed by the Table 8.

<table>
<thead>
<tr>
<th></th>
<th>Acute myocardial infarction</th>
<th>&lt;B&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
<td>Total</td>
</tr>
<tr>
<td>Leflunomide</td>
<td>Yes 6</td>
<td>194</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>No 552</td>
<td>5386</td>
<td>5938</td>
</tr>
<tr>
<td>Total</td>
<td>558</td>
<td>5580</td>
<td>6138</td>
</tr>
</tbody>
</table>

k = -0,03888389
p value (k) = 0,0023162

X^2(Excl) = 0,20546147

According to the study of Suissa et al. (Suissa et al., 2006) with a sample size of N = 6138, we accept the Null-hypothesis. Leflunomide and acute myocardial infarction (AMI) are excluding each other (X^2(Excl) = 0.2, k=-0.03, p value(k) = 0.002). Q. e. d.
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Suissa et al. (Suissa et al., 2006) were able to provide evidence that the use of leflunomide is associated with a reduction in AMI in patients with RA while AMI (i.e., CAD) itself is caused by HCMV (Barukčić, 2018d). Conclusio. Leflunomide is highly effective against HCMV (Waldman et al., 1999; Sudarsanam et al., 2006; Verkaik et al., 2013; Lu et al., 2015). Glioblastoma multiforme is caused by HCMV. Therefore, to date it is more than necessary that leflunomide becomes part of a therapy against glioblastoma multiforme.

3.4. Etoricoxib and glioblastoma multiforme.
Etoricoxib is an anti-rheumatic drug used to help to control the inflammatory process in rheumatoid arthritis. Etoricoxib can be associated with an increased or decreased risk of coronary artery disease (CAD) in rheumatoid arthritis (RA) patients.

Claims.
Null hypothesis: Etoricoxib and coronary artery disease (CAD) are excluding each other.

Alternative hypothesis: Etoricoxib and coronary artery disease (CAD) are not excluding each other.
The significance level (Alpha) below which the null hypothesis will be rejected is alpha=0.05.

Proof.
Hung et al. (Hung et al., 2017) investigated whether an anti-rheumatic drug like etoricoxib is associated with an increased risk of coronary artery disease (CAD) in rheumatoid arthritis (RA) patients. The data of the study of Hung et al. are viewed by the Table 9.

Table 9. The study of Hung et al. 2017

<table>
<thead>
<tr>
<th>Etoricoxib</th>
<th>CAD &lt;B&gt;</th>
<th>Yes</th>
<th>No</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>12</td>
<td>144</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1241</td>
<td>4863</td>
<td>6104</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1253</td>
<td>5007</td>
<td>6260</td>
<td></td>
</tr>
</tbody>
</table>

k = -0.04923852
p value (k) = 9.7891E-05

<A> EXCLUDES <B> and vice versa
p(EXCL) = 0.998083067
X^2(EXCL) = 0.9533031

The use of etoricoxib and CAD are excluding each other. The study of Hung et al. (Hung et al., 2017) with a sample size of N = 6260 provided significant evidence that etoricoxib is effective against CAD. We accept the Null-hypothesis: Etoricoxib and coronary artery disease (CAD) are excluding each other (X^2(Excl) = 0.9533031, k = -0.04, p value = 9.7891E-05). Q.e.d.

Based on the data of Hung et al. (Hung et al. 2017), etoricoxib is an anti-dot and effective against CAD. CAD itself is caused by HCMV (Barukčić, 2018d). Conclusio. Etoricoxib is effective against HCMV. Glioblastoma multiforme is caused by HCMV. Therefore, etoricoxib is of use for the therapy of glioblastoma multiforme.

4. Discussion
Due to the conflicting reports concerning the presence of the human cytomegalovirus in glioma tissue the association between human cytomegalovirus (HCMV) infection and glioblastoma is still a source of debate. While some studies detected HCMV DNA, RNA, and proteins et cetera in GBM tissues others studies (Taha et al., 2016) have not. In most of the previous studies, only a very restricted number of HCMV viral targets with different sensitive techniques (Immunglobulin G antibodies, immunohistochemical analysis, PCR, in situ hybridization, immunohistochemistry, real-time PCR et cetera) accompanied by a very different personal skill were analysed. Thus, the question is justified to which extent was the entire viral genome present when detected.
Human cytomegalovirus is the cause of glioblastoma multiforme

5. Conclusion
Human cytomegalovirus is the cause of glioblastoma multiforme

6. Acknowledgments
Dedicated to Celina. Celina, may this article be of help for you.

7. References


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