Human cytomegalovirus (HCMV) infection has been supposed to play an important role in the pathogenesis of human atherosclerosis (AS). Although many authors proved the presence of viral DNA in arterial wall tissue, the role of HCMV in the origin and progress of atherosclerosis still remains unclear and no definite consensus has been reached. Whether HCMV may be involved in the development of AS has not yet been established.

Methods: The purpose of this study was to investigate whether HCMV and AS are causally related. The conditio sine qua non method, the conditio per quam method, the causal relationship and other methods were used to re-analyzed the data available.

Results: HCMV is a necessary condition of AS. HCMV is a sufficient condition of AS. There is a highly significant cause effect between a HCMV infection and AS. This review and meta-analysis results provide striking evidence that a HCMV infection and AS are causally connected.

Conclusions: In conclusion, a HCMV infection is the cause of AS.

Keywords: Human cytomegalovirus, atherosclerosis, causal relationship.
1. Introduction

Atherosclerosis is as old as human (Kälvegren, 2007) mankind itself while the term *atheroma* has been coined by *Celsius* (Cottet & Lenoir, 1992) more than two thousands of years ago. However, it was especially *Lobstein* (Lobstein, 1833) who defined in 1833 the word *atheromatosis*. In 1904, Félix Jacob Marchand (1846 – 1928) renamed the word “atheroma” by the word “atherosclerosis” (Marchand, 1904, pp. 23–59). The historical roots of a scientific understanding of atherosclerosis can already be found in pre-modern and medieval age. Historically, it was *Albrecht von Haller* who described in 1755 atherosclerosis as a degenerative (Haller, 1755) process observed in the intima of arteries (Haller, 1755) while *John Hunter* (1728–1793), the famous Scottish physician and the “Founder of Scientific Surgery” (Androutsos, Vladimiro, & Diamantis, 2007) observed already in 1793 that inflammation (Wilson, 1793) of the internal surface of veins is common. In the following, the British surgeon *Joseph Hodgson* famous for his 1815 monograph (Hodgson, 1815) was of the opinion that inflammation (Hodgson, 1815) was the underlying cause of atheromatous arteries. The inflammatory theory of atherosclerosis was advocated in 1856 by the prominent German pathologist *Rudolf Virchow* too who writes about “*die acute Entzüdung der Arterien*” (Virchow, 1856) proposing an ‘infiltration’ theory of *atherosclerosis* claiming that atherosclerosis is a chronic inflammatory disease of the intima of an artery. In point of fact, it is notable that since the 19th century several authors postulated that the development of atherosclerotic plaques and their rupture is determined by an inflammation (Huchard, 1891) caused by infection (Gilbert & Lion, 1889). In the following, several different infectious agents have been implicated in the etiology of atherosclerosis including *C. pneumoniae*, *H. pylori* and other and the development and progression has kept growing and includes several viral infections too. Minick et al. (Minick, Fabricant, Fabricant, & Litrenta, 1979) performed an experimental study in 1979, while contaminating birds by a herpesvirus. The birds developed typical atherosclerosis. Among all human herpesviruses (HHV), especially human herpesvirus 5 (HHV-5) or *human cytomegalovirus* has been linked with the development of atherosclerosis. The HCMV infection is relatively common among women of reproductive age, the seroprevalence is ranging from 45 to 100% (Cannon, Schmid, & Hyde, 2010) while the worldwide HCMV...
seroprevalence (Mussi-Pinhata et al., 2018) shows a substantial geographic variation. In point of fact, the overall seroprevalence rate of HCMV increases gradually from 36.3% in 6-11-year-olds to 90.8% in those aged > or =80 years (Staras et al., 2006), while the seroprevalence among women of reproductive age is about 45–100%. Increasing arguments supports a direct link between HCMV infection and cardiovascular disorders, stroke et cetera and are documented by evaluation of anti-HCMV antibodies, PCR analysis and other studies. Mounting but to some extent still conflicting (Ridker, Hennekens, Stampfer, & Wang, 1998) evidence strongly indicates (Simanek et al., 2011) the implication of persistent HCMV infection with several health-related changes including atherosclerosis. Findings indicate that even relatively young asymptomatic individuals seropositive for CMV have abnormal endothelial dysfunction (Grahame-Clarke et al., 2003). However, contradictory results have also been reported too and more a detailed review and meta-analysis is needed before the final verdict on this exciting question can be presented and the more popular “the lipid hypothesis” (I. Barukčić, 2019e; Linton et al., 2000) of arteriosclerosis became, the less the infection hypothesis of arteriosclerosis became important over time. To date, atherosclerosis is the most frequent reason of deaths in Western countries and equally an important problem of the contemporary medicine. However, despite the long history of investigation, a cause or the cause of atherosclerosis remains largely unknown.

2. Material and methods

HCMV is a double-stranded DNA virus of the β-herpesvirus family genome and persists in certain human host cells for life after primary infection (Dolan et al., 2004), HCMV is never cleared by human host. Reactivation and latency are defining characteristics of HCMV infection. A reactivation from latency (Sinclair & Sissons, 2006) even in non-immunocompromised individuals can results in serious disease. HCMV IgG indicates HCMV positivity or latency while changes of HCMV IgG during HCMV latency might point to recent or frequent HCMV reactivation. Reactivations or superinfections may result in higher titers of HCMV immunoglobulin G (IgG) antibodies but of increased levels of pro-inflammatory markers too. HCMV-specific IgG is used as an indicator for long-term HCMV infection. HCMV IgG titers are measured while using different kits. The cutoff value for HCMV
positivity was different. The sensitivity and specificity of these kits is different which might have impact on the results achieved.

2.1. Material

2.1.1. Search Strategy

In general, for the questions addressed in this paper, the electronic database PubMed was searched for appropriate studies conducted in any country which investigated the relationship between HCMV and AS 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 “cytomegalovirus and atherosclerosis”. Those articles were considered for a re-view where data were available without significant access barrier. Additionally, the reference list of identified articles was used as a potential source of articles appropriate for this study.

Table 1. The article selection process of the studies analyzed

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<tr>
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<tr>
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<td><strong>4. Included</strong></td>
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<tr>
<td>Articles included in the meta-analysis</td>
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</tbody>
</table>

Adopted from PRISMA 2009 (Moher, Liberati, Tetzlaff, & Altman, 2009).
2.1.2. HCMV IgG-Studies considered for re-analysis

The following CMV IgG sero-epidemiological studies (Adam et al., 1987; Adler, Hur, Wang, & Vetrovec, 1998; Blum, Peleg, & Weinberg, 2003; Gabrylewicz et al., 2003; González-Quijada, Mora-Simón, & Martin-Ezquerro, 2014; Huang et al., 2012; Kurkowska-Jastrzebska et al., 2016; Li, Xu, & Wang, 1996; Loebe et al., 1990; Mundkur et al., 2012; Ossewaarde, Feskens, De Vries, Vallinga, & Kromhout, 1998; Pesonen et al., 2009; Ridker et al., 1998; Safaie, Ghotaslou, & Montazer Ghaem, 2010; Sepúlveda, Moreu, Cantón, Pajin, & Rodríguez, 1999; Timóteo et al., 2003; Yang et al., 2018; Zhang et al., 2015; Zhu, Quyyumi, Norman, Csako, & Epstein, 1999) as presented by Table 2 were considered for meta-analysis.

Table 2. Without HCMV IgG sero-positivity no AS.

| Study            | Year | n    | a    | a+c  | b    | b+d  | k    | P Value | p(SINE) | X²(SINE|Bt) | p(SINE) | p(IOI) | p(IOU) | p(IOI) | p(IOU) |
|------------------|------|------|------|------|------|------|------|---------|---------|--------|---------|--------|--------|--------|--------|
| Kurkowska-Jastrzębska et al. | 2016 | 195  | 114  | 116  | 73   | 79   | 0.145| 0.041   | 0.990   | 0.010  | 0.034   | 0.918  | 0.554  | 0.364  |
| Adam et al.      | 1987 | 314  | 141  | 157  | 116  | 157  | 0.207| 0.000   | 0.949   | 0.050  | 1.631   | 0.637  | 0.318  | 0.318  |
| Izadi et al.     | 2012 | 105  | 30   | 33   | 60   | 72   | 0.101| 0.151   | 0.971   | 0.028  | 0.273   | 0.714  | 0.171  | 0.543  |
| Izadi et al.     | 2012 | 105  | 30   | 33   | 60   | 72   | 0.101| 0.151   | 0.971   | 0.028  | 0.273   | 0.714  | 0.171  | 0.543  |
| Mundkur et al.   | 2012 | 866  | 425  | 433  | 422  | 433  | 0.024| 0.145   | 0.991   | 0.009  | 0.148   | 0.956  | 0.478  | 0.478  |
| Huang et al.     | 2012 | 400  | 197  | 200  | 195  | 200  | 0.036| 0.220   | 0.993   | 0.007  | 0.045   | 0.960  | 0.480  | 0.480  |
| Safaie et al.    | 2010 | 157  | 94   | 113  | 28   | 44   | 0.211| 0.006   | 0.879   | 0.114  | 3.195   | 0.554  | 0.497  | 0.057  |
| Gabrylewicz et al. | 2003 | 158  | 94   | 110  | 15   | 48   | 0.539| 0.000   | 0.899   | 0.096  | 2.327   | 0.392  | 0.386  | 0.006  |
| Blum et al.      | 2003 | 91   | 57   | 60   | 25   | 31   | 0.228| 0.032   | 0.967   | 0.032  | 0.150   | 0.802  | 0.560  | 0.242  |
| Timóteo et al.   | 2003 | 90   | 57   | 60   | 24   | 30   | 0.236| 0.029   | 0.967   | 0.033  | 0.150   | 0.800  | 0.567  | 0.233  |
| Li et al.        | 1996 | 186  | 101  | 106  | 68   | 80   | 0.177| 0.012   | 0.973   | 0.027  | 0.236   | 0.817  | 0.478  | 0.339  |
| Loebe et al.     | 1990 | 50   | 20   | 26   | 6    | 24   | 0.519| 0.000   | 0.880   | 0.313  | 1.385   | 0.040  | 0.040  | 0.000  |
| **Total**        | **2717** | **1360** | **1447** | **1092** | **1270** | **0.968** | **0.024** | **9.846** | **0.692** | **0.392** | **0.300** |

Alpha = 0.05

D. f. = 12

X²(Critical) = 21.0261

P Value (right-tail) = 0.6295

The study design of the most studies was very inappropriate thus that the result of the re-analysis can be biased. The only study design which was convincing was the study design of Loebe et
with \( p(IOI) + p(IOU) = 0.040 \). Only studies with \( p(IOI) < 0.367 \) were able to provide evidence of a significant cause effect relationship.

### 2.1.3. HCMV IgG-Studies not considered for re-analysis

It was not possible to consider several CMV IgG sero-epidemiological studies (Al-Ghamdi, 2012; Altanavch, Roubalová, Broz, Hrubá, & Anděl, 2003; Betjes, Litjens, & Zietse, 2007; Bloemenkamp et al., 2002; Blum et al., 1998; Cai, Cai, & Lu, 2003; Elkind et al., 2010; Eryol et al., 2005; Espinola-Klein et al., 2002; Gkrania-Klotsas et al., 2012; Grahame-Clarke et al., 2003; Gredmark, Jonasson, van Gosliga, Ernerudh, & Söderberg-Nauclér, 2007; Jeong et al., 2015; Jha & Mittal, 2009; Jha, Prasad, & Mittal, 2008; Kawasaki et al., 2016; Knudsen et al., 2019; Laek et al., 2013; Lidón et al., 2019; Liu et al., 2011; Loebe et al., 1990; López de Atalaya, Cour, García, Ferro, & Perezagua, 1989; Martínez-Rodríguez et al., 2013; Masiá et al., 2013; Musiani et al., 1990; Olson et al., 2013; Rabczyński et al., 2015; Rabczyński, Jakobsche, & Adamiec, 2007; Rajasekhar et al., 2002; Rothenbacher et al., 2003; Siennicka, Kruk, Przyłuski, & Krajewski, 2001; Simanek et al., 2011; Sorlie et al., 1994; Szklo et al., 2009; Tewari, Nijhawan, Mishra, Dudeja, & Salopal, 2012; Tracy et al., 2013; Visseren et al., 1997; Voorend, van der Ven, Kubat, Lodder, & Bruggeman, 2008; Witherell et al., 2003; Zhang et al., 2015) for meta-analysis due to various reasons (data access barriers, data are self-contradictory et cetera).

### 2.1.4. HCMV is a sufficient condition of AS

Polymerase chain reaction (PCR) and other different HCVM DNA based studies where considered for a re-analysis. The PCR methodology itself is not completely free of any errors and it is not possible to exclude any imponderability due to PCR. HCMV DNA must be purified from a specimen with different quality while using a certain kit. Manufacturer's protocol does not guarantee a PCR specify and sensitivity of 100 %. HCMV DNA must be amplified by PCR using different (forward and reverse) HCMV primers selected from a certain region of the CMV genome (Table 3).
### Table 3. HCMV (PCR DNA) is a sufficient condition of AS

| Study       | Year | n  | a/c | b/d | k   | Value | p (IMP) | P Value | X² (IMP| At) | X² (IMP| Bt) | p (IOU) | p (IOI) |
|-------------|------|----|-----|-----|-----|-------|---------|---------|--------|-------|-------|---------|---------|
| Cao et al.  | 2017 | 40 | 21  | 25  | 2   | 15    | 0.692   | 0.000   | 0.950  | 0.049 | 0.174 | 0.267   | 0.200   | 0.050   |
| Wang et al. | 2016 | 32 | 14  | 15  | 0   | 17    | 0.939   | 0.000   | 1.000  | 0.000 | 0.000 | 0.000   | 0.094   | 0.031   |
| Beyaz et al.| 2019 | 36 | 12  | 19  | 0   | 17    | 0.669   | 0.000   | 1.000  | 0.000 | 0.000 | 0.000   | 0.139   | 0.194   |
| Izadi et al.| 2012 | 87 | 37  | 48  | 18  | 39    | 0.319   | 0.002   | 0.793  | 0.187 | 5.891 | 8.308   | 0.184   | 0.080   |
| Yi et al.   | 2008 | 55 | 21  | 35  | 6   | 20    | 0.289   | 0.024   | 0.891  | 0.103 | 1.333 | 1.800   | 0.127   | 0.145   |
| Ibrahim et al.| 2005 | 96 | 5   | 48  | 0   | 48    | 0.234   | 0.028   | 1.000  | 0.000 | 0.000 | 0.000   | 0.448   | 0.448   |
| Heybar et al.| 2015 | 110| 8   | 55  | 2   | 55    | 0.190   | 0.039   | 0.982  | 0.018 | 0.400 | 0.073   | 0.409   | 0.049   |
| Izadi et al.| 2014 | 60 | 9   | 30  | 1   | 30    | 0.358   | 0.006   | 0.983  | 0.017 | 0.100 | 0.033   | 0.333   | 0.333   |
| Yi et al.   | 2008 | 55 | 21  | 35  | 6   | 20    | 0.289   | 0.024   | 0.891  | 0.103 | 1.333 | 1.800   | 0.127   | 0.145   |
| Bayram et al.| 2011 | 60 | 3   | 30  | 0   | 30    | 0.229   | 0.119   | 1.000  | 0.000 | 0.000 | 0.000   | 0.450   | 0.450   |
| Imbronito et al.| 2010 | 78 | 28  | 30  | 0   | 48    | 0.947   | 0.060   | 1.000  | 0.000 | 0.000 | 0.000   | 0.256   | 0.026   |
| Gredka et al.| 2009 | 25 | 21  | 22  | 0   | 3     | 0.846   | 0.002   | 1.000  | 0.000 | 0.000 | 0.000   | 0.720   | 0.040   |
| Reszka et al.| 2008 | 60 | 22  | 40  | 10  | 20    | 0.047   | 0.202   | 0.833  | 0.154 | 3.125 | 5.000   | 0.200   | 0.133   |
| Westphal et al.| 2006 | 116| 52  | 68  | 0   | 48    | 0.757   | 0.000   | 1.000  | 0.000 | 0.000 | 0.000   | 0.034   | 0.138   |
| Shi et al.  | 2002 | 33 | 4   | 10  | 1   | 23    | 0.457   | 0.020   | 0.970  | 0.030 | 0.200 | 0.043   | 0.545   | 0.152   |
| Hu et al.   | 2001 | 90 | 51  | 60  | 2   | 30    | 0.750   | 0.089   | 0.878  | 0.022 | 0.075 | 0.133   | 0.256   | 0.078   |
| Hendrix et al.| 1990 | 64 | 27  | 30  | 18  | 34    | 0.405   | 0.001   | 0.719  | 0.245 | 7.200 | 9.529   | 0.172   | 0.234   |
| Lin et al.  | 2003 | 224| 64  | 200 | 2   | 24    | 0.161   | 0.008   | 0.991  | 0.009 | 0.061 | 0.167   | 0.188   | 0.598   |
| Radic et al.| 2001 | 101| 16  | 53  | 0   | 48    | 0.413   | 0.000   | 1.000  | 0.000 | 0.000 | 0.000   | 0.317   | 0.366   |
| Horvith et al.| 2000 | 331| 185 | 244 | 0   | 87    | 0.672   | 0.000   | 1.000  | 0.000 | 0.000 | 0.000   | 0.296   | 0.178   |
| Chiu et al. | 1997 | 96 | 27  | 76  | 0   | 20    | 0.321   | 0.001   | 1.000  | 0.000 | 0.000 | 0.000   | 0.073   | 0.510   |
| Chen et al. | 1995 | 47 | 13  | 32  | 1   | 15    | 0.346   | 0.015   | 0.979  | 0.021 | 0.071 | 0.067   | 0.021   | 0.383   |

**Total** 1896 661 1205 69 691 0.964 0.036 19,964 27,220 0.254 0.233

Alpha = 0.05

D. f. = 22

X² (Critical) = 33.9244

P Value (right-tail) = 0.5853 0.2030
2.1.5. HCMV is a necessary condition of AS

Ten HCMV PCR DNA studies were able to provide evidence of a condition sine qua non relationship between HCMV and AS (Table 3).

Table 4. HCMV (PCR DNA) is a necessary condition of AS

| Study              | Year | n   | a  | a+c | b  | b+d | k   | Value | p(SINE) | X²(SINE|Br) | X²(SINE|At) | p(IOU) | p(IOI) |
|--------------------|------|-----|----|-----|----|-----|-----|-------|---------|--------|---------|--------|--------|
| Cao et al.         | 2017 | 40  | 21 | 25  | 2  | 15  | 0,692| 0,000 | 0,900   | 0,941   | 0,200   | 0,050  |
| Wang et al.        | 2016 | 32  | 14 | 15  | 0  | 17  | 0,939| 0,000 | 0,969   | 0,031   | 0,067   | 0,056  | 0,094  | 0,031  |
| Beyaz et al.       | 2019 | 36  | 12 | 19  | 0  | 17  | 0,669| 0,000 | 0,806   | 0,177   | 2,579   | 2,042  | 0,139  | 0,194  |
| Izadi et al.       | 2012 | 87  | 37 | 48  | 18 | 39  | 0,319| 0,002 | 0,874   | 0,119   | 2,521   | 3,781  | 0,184  | 0,080  |
| Imbronito et al.   | 2010 | 78  | 28 | 30  | 0  | 48  | 0,947| 0,000 | 0,974   | 0,025   | 0,133   | 0,080  | 0,256  | 0,026  |
| Gred.-Russ et al.  | 2009 | 25  | 21 | 22  | 0  | 3   | 0,846| 0,002 | 0,960   | 0,045   | 0,250   | 0,720  | 0,040  |
| Westphal et al.    | 2006 | 116 | 52 | 68  | 0  | 48  | 0,757| 0,000 | 0,862   | 0,129   | 3,765   | 4,000  | 0,034  | 0,138  |
| Shi et al.         | 2002 | 33  | 4  | 10  | 1  | 23  | 0,457| 0,020 | 0,818   | 0,166   | 3,600   | 1,286  | 0,545  | 0,152  |
| Hu et al.          | 2001 | 90  | 51 | 60  | 2  | 30  | 0,750| 0,000 | 0,900   | 0,095   | 1,350   | 2,189  | 0,256  | 0,078  |
| Hendrix et al.     | 1990 | 64  | 27 | 30  | 18 | 34  | 0,405| 0,001 | 0,953   | 0,046   | 0,300   | 0,474  | 0,172  | 0,234  |
| Total              | 601  | 327 | 41 | 274 | 601| 267 | 0,900| 0,593 | 15,000  | 15,098  | 0,260   | 0,102  |

Alpha = 0,05
D. f. = 10
X²(Critical) = 18,3070
P Value (right-tail) = 0,1321 0,1285

The studies of Wang et al., Imbronito et al. and Westphal et al. did not provide an appropriate control group. Still, the calculation of the Chi-square statistics was possible, a fair study design provided. The following PCR and other HCMV DNA studies (Chen et al., 2003; Ciervo, Mancini, Sale, Russo, & Cassone, 2008; Courivaud et al., 2013; Hagiwara et al., 2007; Horváth, Cerný, Benedik, Hökl, & Jelinková, 2000; Huang et al., 2012; Izadi et al., 2012; Kilic et al., 2006; Latsios, Saetta, Michalopoulos, Agapitos, & Patsouris, 2004; Lebedeva, Shpektor, Vasilieva, & Margolis, 2018; Lee et al., 2014; Lin, Chen, Chen, Wang, & Eng, 2003; Melnick, Adam, & DeBakey, 1990; Melnick, Hu, Burek, Adam, & DeBakey, 1994; Nyberg, Skagius, Nilsson, Ljungh, & Henriksson, 2008; Pinar et al., 2004; Priyanka, Kaarthikeyan, Nadathur,
Mohanraj, & Kavarthapu, 2017; Radke et al., 2001; Reinhardt et al., 2003; Reszka et al., 2008; Saetta, Fanourakis, Agapitos, & Davaris, 2000; Shi & Tokunaga, 2002; Skowronski, Mendoza, Smith, & Jaski, 1993; Tremolada et al., 2011; Watt, Aesch, Lanotte, Tranquart, & Quentin, 2003; Westphal et al., 2006; Xenaki, Hassoulas, Apostolakis, Sourvinos, & Spandidos, 2009; Yamashiroya, Ghosh, Yang, & Robertson, 1988) were not considered for further analysis due to several reasons.

### 2.1.6. Statins and AS

The statin drug studies were not able to establish evidence of the lipid hypothesis of beyond all doubt (I. Barukčić, 2019e).

### 2.1.6. Drugs and AS

Under the assumption that atherosclerosis of coronary arteries (CAD) is an inflammatory process, an ‘immunosuppressive’ or ‘immunomodifying’ therapy in patients treated with 'immunosuppressive' or modifying medication and other drugs should decrease the number of new cardio-vascular events (CAD incidence). It was possible to identify view studies (Hung et al., 2017; Suissa, Bernatsky, & Hudson, 2006; Wu et al., 2016) which investigated the relationship between intake of putative immunosuppressive drugs and cardio-vascular events.

**Table 5. Drugs and cardio-vascular events**

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<tr>
<td>Suissa et al.</td>
<td>2006</td>
<td>Leflunomide</td>
<td>6138</td>
<td>6</td>
<td>558</td>
<td>194</td>
<td>5380</td>
<td>-0.03808</td>
<td>0.00038</td>
<td>0.99999</td>
<td>0.00098</td>
<td>0.88800</td>
<td>0.01452</td>
<td>0.87651</td>
<td>0.05833</td>
</tr>
<tr>
<td>Total</td>
<td>22770</td>
<td>31</td>
<td>3400</td>
<td>656</td>
<td>19560</td>
<td>0.9986</td>
<td>0.00014</td>
<td>1.6145</td>
<td>0.5323</td>
<td>0.8228</td>
<td>0.1105</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Alpha = 0.05
D. f. = 4
Χ²(Critical) = 9.4877
A study design which aims to investigate an \textit{exclusion relationship} should assure conditions where $p(\text{IOI}) = 0$ or as near to zero as possible. Especially Wu et al. and Suissa et al. assured appropriate conditions but Hung et al. only to some extent too. The etoricoxib analysis of Thöne et al. (Thöne, Kollhorst, & Schink, 2017) and of Masclee et al. (Masclee et al., 2018) was not considered for a re-analysis.

\section*{2.2. Methods}

### 2.2.1. Definitions

\textit{Definition 1. (The 2x2 Table)}

Karl Pearson (K. Pearson, 1904) introduced in 1904 the notion of a contingency table (I. Barukčič, 2019a, 2019d) or two by two table. Especially the relationships between Bernoulli (i.e. Binomial) distributed random variables can be examined by contingency tables. Thus far, let a Bernoulli distributed random variable $A_t$ occur/exist et cetera with the probability $p(A_t)$ at the Bernoulli trial (period of time) $t$. Furthermore, let another Bernoulli distributed random variable $B_t$ occur/exist et cetera with the probability $p(B_t)$ at the \textit{same} Bernoulli trial (period of time) $t$. Let $p(a_t) = p(A_t \cap B_t)$ denote the joint probability distribution of $A_t$ and $B_t$ at the \textit{same} Bernoulli trial (period of time) $t$. The following table (\textbf{Table 8}) may show the relationships in more details.

\begin{table}[h]
\centering
\begin{tabular}{lccc}
\hline
\textbf{Condition A} & \textbf{Conditioned} & \textbf{B} & \\
\hline
\textbf{Yes} & \textbf{Yes} & \textbf{+1} & \\
& $p(a_t)$ & $p(b_t)$ & $p(A_t)$ \\
\textbf{No} & \textbf{No} & \textbf{+0} & \\
& $p(c_t)$ & $p(d_t)$ & $p(A_t)$ \\
\textbf{Total} & & & 1 \\
\hline
\end{tabular}
\caption{The probabilities of a contingency table}
\end{table}

In this context, it is \textit{per definitionem}
The meaning of the abbreviations $a$, $b$, $c$, $d$, $n$ et cetera are explained by following 2 by 2 contingency table (Table 7). The relationships are valid even under conditions where $n = 1$.

**Table 7. The sample space of a contingency table**

<table>
<thead>
<tr>
<th>Conditioned B</th>
<th>(Outcome)</th>
<th>Yes = +1</th>
<th>No = +0</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Condition A</strong></td>
<td>Yes = +1</td>
<td>a</td>
<td>b</td>
<td>A</td>
</tr>
<tr>
<td>(risk factor)</td>
<td>No = +0</td>
<td>c</td>
<td>d</td>
<td>A</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td>B</td>
<td></td>
<td>$n$</td>
</tr>
</tbody>
</table>
**Definition 2. (Index of unfairness)**

The index of unfairness (IOU) is defined (I. Barukčić, 2019c) as

$$IOU \equiv \left( \frac{A + B}{n} \right) - 1$$

(3)

The range of $A$ is $0 \leq A \leq n$, while the range of $B$ is $0 \leq B \leq n$. A study design based on $A=B=0$ leads to an index of unfairness of $IOU = (((0+0)/n)-1) = -1$. A study design which demands that $A=B=n$ leads to an index of unfairness of $IOU = (((n+n)/n)-1) = +1$. In particular, the range of the index of unfairness is $[-1;+1]$.

**Definition 3. (The study design for single risk factors or conditions)**

Assuming the necessary condition (I. Barukčić, 2018d, 1989, 1997, 2017, 2018a, 2018b, 2019d; K. Barukčić & Barukčić, 2016, 2016) relationship (*conditio sine qua non*) is given in the population ($a + b + d = n$), it has to be that $c = 0$ or

$$B - a \equiv n - A - d = c = 0$$

$$A + B \equiv n + a - d$$

$$\frac{A + B}{n} \equiv \left( \frac{n}{n} \right) + \left( \frac{+a - d}{n} \right)$$

$$\left( \frac{A + B}{n} \right) - 1 \equiv \left( \frac{+a - d}{n} \right)$$

$$\left( \frac{A + B}{n} \right) - 1 \equiv \left( \frac{+a - d}{n} \right) = IOU$$

(4)

A study design which assures an index of unfairness as near as possible to $IOU = 0$ or $a=d$ is appropriate enough to recognize a single risk factor or single condition like *conditio sine qua non* or *conditio per quam* but is not appropriate enough to recognize an exclusion (I. Barukčić, 2019e) relationship.

**2.2.2. Data analysis**

Barukčić, 2016a, 2018b, 2018a, 2019d; K. Barukčić & Barukčić, 2016; K. Barukčić, Barukčić, & Barukčić, 2018), at every single Bernoulli trial (Uspensky, 1937, p. 45) and was used to proof the data for a causal relationship while the significance was tested by the hypergeometric distribution (HGD) and sometimes by the chi-square distribution (Karl Pearson, 1900) too. The conditio sine qua non (I. Barukčić, 2018d, 1989, 1997, 2017, 2018a, 2018b, 2019d; K. Barukčić & Barukčić, 2016, 2016) relationship (SINE) was used to proof the hypothesis, without (I. Barukčić, 2019e) HCMV infection no AS. The conditio per quam (I. Barukčić, 2018d, 1989, 1997, 2017, 2018a, 2018b, 2019d; K. Barukčić & Barukčić, 2016, 2016) relationship (IMP) was used to proof the hypothesis, if (I. Barukčić, 2019e) HCMV infection then AS. The necessary and sufficient condition (I. Barukčić, 2018d, 1989, 1997, 2017, 2018a, 2018b, 2019d; K. Barukčić & Barukčić, 2016, 2016) relationship (SINE) can be used to proof the hypothesis, (without HCMV infection no AS) and (if HCMV infection then AS). The index of unfairness (I. Barukčić, 2019c) and the index of independence (I. Barukčić, 2019b) was used to control publication bias. All statistical analyses were performed with Microsoft® Excel® for Mac® version 16.2 (181208) software (© 2018, Microsoft GmbH, Munich, Germany). The level of significance was set to 0.05.

3. Results

Theorem 1. Without HCMV IgG sero-positivity no AS

Claim.

Null-Hypothesis: HCMV IgG sero-positivity is a necessary condition of AS.

Alternative Hypothesis: HCMV IgG sero-positivity is not a necessary condition of AS.

Proof.

In toto, 12 studies with a sample size of n = 2717 (Table 2) were considered for a re-analysis of a conditio sine qua non relationship between HCMV and AS based on HCMV IgG serology. The study design was not highly appropriate (Mean (IOU) = 0.392; Mean (IOI) = 0.300). In this context, the data analyzed could be of very limited use. However, the average conditio sine qua non relationship between HCMV and AS was p(SINE) = 0.968. The X² calculated was determined as X²(Calculated) = 9.846 while the X² critical (degrees of freedom = 12; Alpha =
0,05) was found to be $X^2(\text{Critical}) = 21,0261$. Since $X^2(\text{Calculated}) < X^2(\text{Critical})$ it was not possible to refute the null-hypothesis. Thus far, we refute the alternative hypothesis and accept the null-hypothesis: HCMV IgG sero-positivity is a necessary condition of AS. In other words, without HCMV IgG sero-positivity no AS.

**QUOD ERAT DEMONSTRANDUM.**

**THEOREM 2. WITHOUT HCMV PCR DNA POSITIVITY NO AS**

**CLAIM.**

Null-Hypothesis: HCMV PCR DNA positivity is a necessary condition of AS.

Alternative Hypothesis: HCMV PCR DNA positivity is not a necessary condition of AS.

**PROOF.**

In toto, 10 HCMV PCR DNA studies (Table 4) were considered for a re-analysis of a condition sine qua non relationship between HCMV and AS based on the detection of HCMV DNA in vessels or plaques but not in serum or plasma. The study design was more or less appropriate (Mean (IOU) = 0,26; Mean (IOI) = 0,102). In this context, the data analyzed were of use even if the average condition sine qua non relationship between HCMV and AS was $p(\text{SINE}) = 0,90$.

The $X^2$ calculated was determined as $X^2(\text{Calculated 1}) = 15,0$ and $X^2(\text{Calculated 2}) = 15,098$ while the $X^2$ critical (degrees of freedom = 10; Alpha = 0,05) was found to be $X^2(\text{Critical}) = 18,307$. Since $X^2(\text{Calculated}) < X^2(\text{Critical})$ it was not possible to refute the null-hypothesis. Thus far, we refute the alternative hypothesis and accept the null-hypothesis: HCMV PCR DNA positivity is a necessary condition of AS. In other words, according to the HCMV PCR DNA studies analyzed, without HCMV PCR DNA positivity no AS.

**QUOD ERAT DEMONSTRANDUM.**
**Theorem 3. If HCMV PCR DNA positivity then AS**

**Claim.**
Null-Hypothesis: HCMV PCR DNA positivity is a sufficient condition of AS.
Alternative Hypothesis: HCMV PCR DNA positivity is not a sufficient condition of AS.

**Proof.**
In toto, 22 HCMV PCR DNA studies presented by Table 3 provided evidence of a sufficient condition relationship between HCMV and AS. The study design was more or less appropriate (Mean (IOU) = 0.254; Mean (IOI) = 0.233) while the sample size of all HCMV PCR DNA studies analyzed was n = 1896. In this context, the data analyzed were of use even if the average conditio per quam relationship between HCMV and AS was only \( p(\text{SINE}) = 0.964 \). The \( X^2 \) calculated was determined as \( X^2(\text{Calculated 1}) = 19.964 \) and as \( X^2(\text{Calculated 2}) = 27.220 \) while the \( X^2 \) critical (degrees of freedom = 22; Alpha = 0.05) was found to be \( X^2(\text{Critical}) = 33.9244 \). Since \( X^2(\text{Calculated}) < X^2(\text{Critical}) \) it was not possible to refute the null-hypothesis. Thus far, we refute the alternative hypothesis and accept the null-hypothesis: HCMV PCR DNA positivity is a sufficient condition of AS. In other words, according to the HCMV PCR DNA studies analyzed, if HCMV PCR DNA positivity then AS.

**Quod erat demonstrandum.**

**Theorem 4. HCMV is the Cause of AS**
The evidence is increasing that HCMV is suspected to initiate and/or to stimulate the process of atherosclerosis too. Thus far, an anti-HCMV drug usage (leflunomide, etoricoxib, etanercept, betahistine (a strong antagonist of the histamine H3 receptor and a weak agonist of the histamine H1 receptor)) could be associated with significantly decreased incidence of atherosclerotic events and would provide some evidence of the infectious etiology of AS. Especially the dose dependent antiviral activity of leflunomide (N-(4′-trifluoromethylphenyl)-5-methylisoxazole-4-carboxamide) against HCMV, an inhibitor of protein kinase activity and pyrimidine synthesis, is known since years (Waldman, Knight, Blinder, et al., 1999; Waldman, Knight, Lurain, et al., 1999). Leflunomide does not to inhibit viral DNA synthesis, but seems to interfere with virion assembly. Meanwhile, there are reports
of efficacy of leflunomide in humans (John, Manivannan, Chandy, Peter, & Jacob, 2004) with HCMV disease too. Gómez Valbuena et al. (Gómez Valbuena, Alioto, Serrano Garrote, & Ferrari Piquero, 2016) administered a patient an initial leflunomide regimen of 100 mg of leflunomide daily for the first five days, followed by 20 mg every 12 hours. After fifteen days of treatment the HCMV viral load had fallen and became undetectable in one month. In the following (four months of treatment) the patient remained with undetectable viral load without having any adverse effect associated with it. To date, a drug-resistant CMV (Tan, 2014) is still a therapeutic challenge. However, even if it has been well confirmed that MicroRNA S25-1 (miR-US25-1) is encoded (Stern-Ginossar et al., 2009) by HCMV to control the life cycle of the virus, today's ability to chemotherapeutically target specific aspects of the HCMV virus life cycle are very limited. In point of fact, single studies provided some indirect evidence, that new and attractive possibilities (Weekes et al., 2013; Wills, Poole, Lau, Krishna, & Sinclair, 2015) in this context should be considered.

CLAIM.

Null-Hypothesis: HCMV is not the cause of AS (k = 0) due to drug studies.

Alternative Hypothesis: HCMV is the cause of AS (k ≠ 0) due to drug studies.

PROOF.

View single drug studies (Etoricoxib, Etanercept, Leflunomide) presented by Table 5 provided some evidence of the infectious hypothesis of atherosclerosis. In this context, it is necessary to point especially to the study of the Suissa et al. group. The study group of Suissa et al. (Suissa et al., 2006) investigated the risk of acute myocardial infarction (AMI) with respect to the use of Leflunomide, a disease-modifying antirheumatic drugs (DMARD) and other medications commonly used in rheumatoid arthritis (RA) and found that acute myocardial infarction rate significantly decreased with the use of any DMARD. The sample size of this study was n = 6138, the index of independence was IOI = 0,05833. The data published by Suissa et al. were appropriate enough to be analyzed for an exclusion relationship. The causal relationship was found to be negative (k = -0,03888; P Value (k) = 0,00038). The exclusion relationship between the use of Leflunomide and acute myocardial infarction was highly significant (p (EXCl) = 0,99902; P Value = 0,00098) while the X² calculated of the exclusion relationship (Table 5)
was determined as $X^2(\text{Calculated} \ 1) = 0,18000$ or as $X^2(\text{Calculated} \ 2) = 0,06452$ while the $X^2$ critical (degrees of freedom = 1; Alpha = 0,05) was found to be $X^2(\text{Critical}) = 3,84145882$. Since $X^2(\text{Calculated}) < X^2(\text{Critical})$ it was not possible to refute the null-hypothesis. Thus far, we refute the alternative hypothesis and accept the null-hypothesis: Leflunomide excludes acute myocardial infarction according the data published by the Suissa et al. group. However, as already discussed previously in greater detail, Leflunomide itself is highly effective against HCMV (Gómez Valbuena, Alioto, Serrano Garrote, & Ferrari Piquero, 2016). Conclusion. The drug studies support the hypothesis that HCMV is the cause of AS.

QUOD ERAT DEMONSTRANDUM.

**THEOREM 5. HCMV IS THE CAUSE OF AS**

**CLAIM.**

Null-Hypothesis: HCMV is not the cause of AS ($k = 0$).

Alternative Hypothesis: HCMV is the cause of AS ($k \neq 0$).

**PROOF.**

The most of the HCMV PCR DNA studies as presented by Table 3 and Table 4 provided a striking evidence of a positive causal relationship between HCMV PCR DNA positivity and AS. The P Value of the causal relationship was calculated by the hypergeometric distribution. As demonstrated by Table 3 and Table 4, HCMV is the cause of AS.

QUOD ERAT DEMONSTRANDUM.

4. Discussion

The lipid hypothesis in the pathogenesis of atherosclerosis (Konstantinov & Jankovic, 2013) is meanwhile more or less refuted (I. Barukčić, 2019e). The results of the HCMV studies re-analyzed in this publication are consistent and do provide convincing evidence of a causal relationship between a HCMV infection and AS. However, better designed studies using more effective assays, study design and methods are needed to resolve this important issue ultimately.
5. Conclusion

This study provides important insights into the mechanisms of HCMV with atherosclerosis. In conclusion, **without** a HCMV infection **no** atherosclerosis (AMI, CHD, Stroke, abdominal aortic aneurysm et cetera). Besides of some limitations of the present study, the facts presented encourage us to conclude that **human cytomegalovirus is the cause of atherosclerosis**. The underlying pathophysiological mechanism linking HCMV with atherosclerosis is yet to be determined in greater detail.

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**Author Contributions**

The author confirms being the sole contributor of this work and has approved it for publication.

**Conflict of Interest Statement**

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. There are no conflict of interest exists according to the guidelines of the International Committee of Medical Journal Editors.

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