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An Unexpected Increase in Adult Appendicitis in England (2000/01 to 2012/13): Could Cytomegalovirus (CMV) be A Risk Factor?

Rodney P. Jones[1*]

[1]*Healthcare Analysis and Forecasting, Camberley, United Kingdom.*

Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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Original Research Article

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ABSTRACT

Aims: To study the trends in admission for diseases of the appendix and to attempt to present a potential basis for the observed (complex) age-dependent trends and etiologies.
Study Design: Longitudinal study of admissions relating to the appendix with analysis by age and gender.
Place and Duration of Study: Admissions for diseases affecting the appendix for the residents of England over the period 2000/01 to 2012/13.
Methodology: Retrospective application of age-standardized admission rates based on 2012/13 as the base year to determine what proportion of the increase in admissions is due to demography or to non-demographic forces. Synthesis of available literature covering diseases of the appendix to propose possible causes for the increase in admissions.
Results: Based on admissions in 2012/13 diseases of the appendix cost the NHS in England around £107 million per annum (roughly £2 per head of population per annum). Admission rates peak at age 17 but have been increasing over the past 14 years in adults but not children. The rate of increase escalates with age and is more rapid in females. The trend for females shows far higher volatility than that for males and both show some degree of cyclic behavior. Depending on age,

**Corresponding author: Email: hcaf_rod@yahoo.co.uk;*

demographic change can only explain between 20% and 40% of the long-term increase. Social and health service factors are unlikely to explain this gap.
Conclusion: An immune/infectious basis for increasing admission rates appears most likely. A possible role for the immune modulating herpes virus, cytomegalovirus (CMV), is discussed in the context of a potential linkage between infection with multiple agents (called the infectious burden) and the development of multiple morbidity. Both of which increase with age and are amenable to manipulation by CMV. The suggested mechanism may also provide insight into why the rates for admission of certain medical diagnoses are increasing far faster than due to demographic change.

Keywords: Diseases of the appendix; fecalith; appendicitis; age; gender; admission rates; time trends; cytomegalovirus; immune function; infectious burden.

1. INTRODUCTION

The rise in emergency admissions, especially those of a medical nature, has been an enduring problem in western health care systems [1-3]. Proposed causes for, and solutions to, the problem mainly revolve around the perceived inability of the health and social care systems to 'manage' demand leading to both preventable and avoidable admissions [1,4-7]. While such problems are real and are amenable to person-based schemes such as integrated health and social care [8], they may not be the ultimate solution to increasing demand due to emerging disease types. Hence the enduring dilemma as to the large discrepancy between the rising actual admission rates over time for specific conditions as opposed to that predicted from demographic-based forecasts [9].

Appendicitis is a common emergency admission with nearly 50,000 consultant episodes in England in 2012/13 at a cost of around £107 million, roughly £2000 per 1000 population. Between 1979 and 1984 in the US, lifetime risk of appendicitis was around (male/female) 8.6% and 6.7% respectively while risk of appendectomy was 12% and 23.1% respectively [10]. However these rates do vary with time. The trends in disease of the appendix has been chosen for this study because it is not dependent on access to a GP, and neither is it a sign of failure to manage demand or of the quality of primary care. Hence we have an indicator disease which does not depend on the supposed efficiency or otherwise of the health or social care systems.

For many years the accepted view was that acute appendicitis originated as the result of an obstruction of the appendix lumen (the inside space of a tubular structure).The appendix then supposedly filled with mucus leading to increasing pressure within the lumen and the walls of the appendix, resulting in thrombosis and occlusion of the small vessels, stasis of lymphatic flow, leading to necrosis and then suppuration. However, more recently evidence has been mounting of a more restricted role for obstruction and a wider role for infectious agents [11-15].

In addition, the incidence of appendicitis has been displaying unexplained trends over time with a general reduction in some countries [16] and in the USA a reduction followed by an increase [13]. After a 22 year career forecasting demand for the purposes of health care activity, capacity and cost planning my single most powerful observation is that many conditions with a medical etiology do not obey the supposed laws of demographic-based forecasting [17-20]. Significant numbers of medical admissions follow patterns which can only be described in terms of infectious outbreaks of both transient (such as influenza, etc) and persistent (such as HIV, all herpes viruses, helicobacter pylori, etc) agents, with seeming knock-on effects against a wide variety of conditions (mainly those which are linked to multimorbidity), especially in the elderly [20].

Throughout the course of their lives, humans acquire infection with multiple pathogens [21] and at the same time develop multiple morbidities [22-23], which according to the observation that many diseases have an infectious origin [24], suggests that the ageing population, now evident in affluent western countries, could be exhibiting an accelerating incidence of particular diseases over and above that predicted from simple demographic change. With over 1,400 known human pathogens [25] it would be logical to assume that there is room for a variety of trends to develop, depending on the infectious sensitivity (with knock-on immune effects) of different conditions and diseases [26].

This study will examine the developing trends from the viewpoint of a Primary Care Organisation (PCO) tasked with containing rising health care costs. It will ask the questions, how much of the increase can be explained by changing age structure, is diagnostic uncertainty a factor and does a valid medical explanation exist for that proportion of the increase which is not due to demography?

2. MATERIALS AND METHODS

2.1 Trends in Admission

Data is from the Health and Social Care Information Centre website for Hospital Episode Statistics (HES) (http://www.hscic.gov.uk/hes). Overall trend in admission for conditions of the appendix was derived from the Healthcare Resource Group (HRG) tabulation. HRG data summarizes all procedures and non-surgical admissions into a number of groups. For data covered by HRG V3, HRG codes F81 to F83 and HRG V4 codes FZ20A, FZ20B, FZ20C, FZ40A, FZ40B, FZ40Z. Admissions coded using V3 were available up to 2011/12 while those coded using V4 were available from 2009/10 to 2012/13. Using the overlap period between the two versions for total diseases of the appendix V4 contains 2.3% more admissions than V3 which is probably due to more sophisticated identification using secondary diagnoses and procedures. The V3 equivalent data for 2012/13 was estimated by aggregation and apportionment of various V4 codes.

2.2 Cost of Admissions in 2012/13

The HRG prices spreadsheet was obtained from the Department of Health website (https://www.gov.uk/government/publications/payment-by-results-pbr-operational-guidance-and-tariffs) and were matched with HRG admissions for 2012/13. Total cost for diseases of the appendix is given in the Introduction section of this paper.

2.3 Admission Rates by Age Band

Admissions by age from 2012/13 HES data for the primary diagnosis as coded by the 10th revision of the International Classification of Diseases (ICD)-10 codes K35 to K38. Matching mid-year population in 2012 for England was from the Office for National Statistics. 2012/13 was the first year in which admissions were broken down into five year age bands and hence the 2012/13 admission rate by age band was then retrospectively applied to mid-year population estimates from 2001 to 2011 to calculate expected versus actual total admissions for the previous year's to demonstrate the contribution from demographic and non-demographic forces over time.

2.4 Role of Gender in Appendix Procedures

Hospital episode statistics data using the Office of Population Census and Surveys Classification of Operations and Procedures (OPCS) v4, procedure codes H01 – H03 (excision of appendix or operation on appendix) were extracted for each gender over the interval 1998/99 to 2012/13.

3. RESULTS AND DISCUSSION

3.1 Trends in Admission

Primary Care Organisations (PCOs) in England pay hospitals based on the HRG code assigned to each admission. HRGs are similar to DRGs used in the USA and other countries. Fig 1 presents the trend for diseases of the appendix which applied for England using V3 of the HRG tariff. Despite the introduction of more numerous V4 HRG in 2009/10, in this instance, V3 has been used simply because data is available for the entire time period. As can be seen a confusing picture emerges of very high growth in appendix procedures for those aged 70 and above, intermediate growth for hospitalization not involving a procedure and highly volatile trends (with growth) for procedures under the age of 70.

In England, PCOs are at the forefront of implementing a wide range of government initiatives designed to contain rising health care costs and to reduce disparities in admission rates. Such confusing trends clearly warrant more detailed analysis to see if they are due to inappropriate hospitalization or if they represent the outcome of other poorly understood forces.

3.2 Effect of Age

Detailed analysis of the trends affecting various diagnoses (within ICD-10 primary diagnosis codes K35 to K38) shows that diverticulum of the appendix (K382), fistula of appendix (K383) and unspecified disease of the appendix (K389) all

showed near constant admissions over the time period, but in combination, only account for less than 0.7% of the total. Unspecified acute appendicitis (K359) declined from 63% to 50% of the total over the time period but was replaced by more precise definitions as coding specificity has improved over time (9 as the last digit in an ICD code indicates the default code in the absence of defining information). For this reason all disease of the appendix have been analyzed as a group. Fig. 1 shows clear evidence for underlying growth and especially in the elderly and the contribution due to age is illustrated in Fig. 2 using four broad age bands.

It is of interest to note that the volatile behavior for the group aged less than 70 observed in Fig. 1 has been decomposed into more age-specific trends. Growth appears to be accelerating as age increases. While this trend has not been age-adjusted, common sense, does however suggest, that the number of persons aged 60 to 74 and 75+ has not doubled in the past 14 years as is observed for the increased number of admissions in those aged over 60 years seen in Fig. 2. As the first step in age-adjusting these trends, admission rates (calculated using 2012/13 data) by age band are shown in Fig. 3, with a peak at 17 and are highest between ages 10 and 24 with a gradual reduction as age increases, i.e. the ageing population should not (in theory) by a major contributor to trends for this condition since sheer weight of numbers implies that those aged under 45 accounted for 76% of admissions in 2012/13.

Using this 2012/13-based calculation of admission rates it is possible to calculate the expected number of admissions for all previous years and this can then be compared to the actual number of admissions. The results of this calculation are presented in Fig. 4 where it can be seen that the effect of age is indeed real. Hence below age 15 admission rates may show a slight reduction of around -0.5 percentage points per annum, however, above age 15 rates of admission are escalating over time up to age around 60 where the increase appears to have diminished. The 95% confidence interval (Poisson) for each of the age bands is ± 2%, ± 1%, ± 4%, ± 6% respectively, which explains some (but not all) of the year-to-year volatility in the individual trend lines but cannot explain the long-term trends.

Hence only an increase of 6.9%, 17.6% and 10.7% respectively for ages 15-59, 60-74 and 75+ is due to demographic forces (data not shown) and demography could only explain 42.6%, 23.9%, 36.4% and 24.6% respectively of the total growth observed in each of the four age bands, i.e. for the older age bands some 65% to 75% of the change is due to unexplained forces (which are therefore driving the rise in costs).

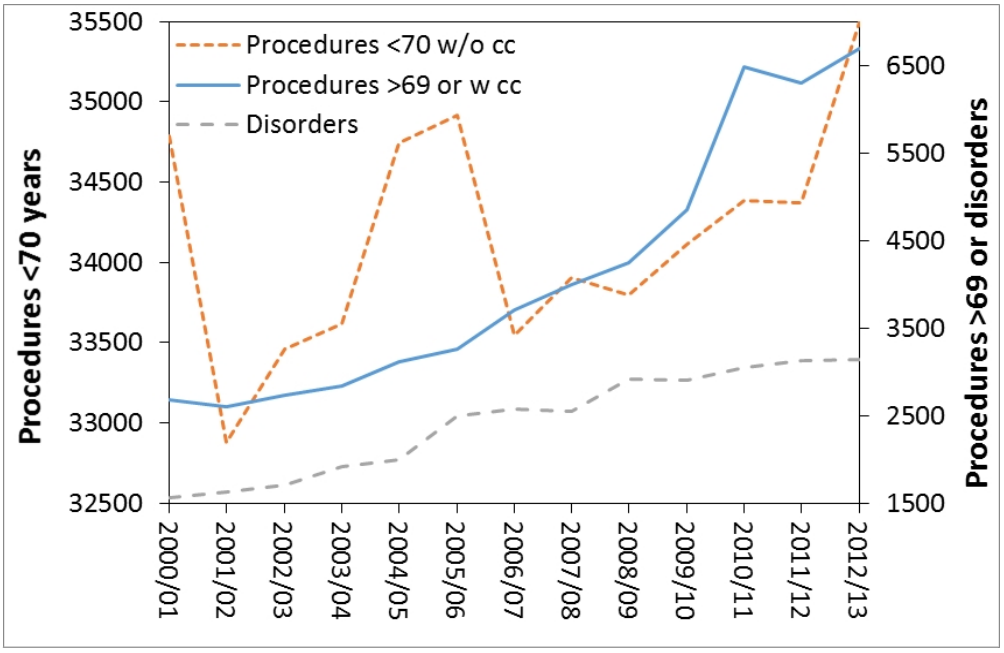

Fig. 1. Trends in diseases of the appendix using V3 of the HRG tariff
w/o cc = without complications, w cc = with complications

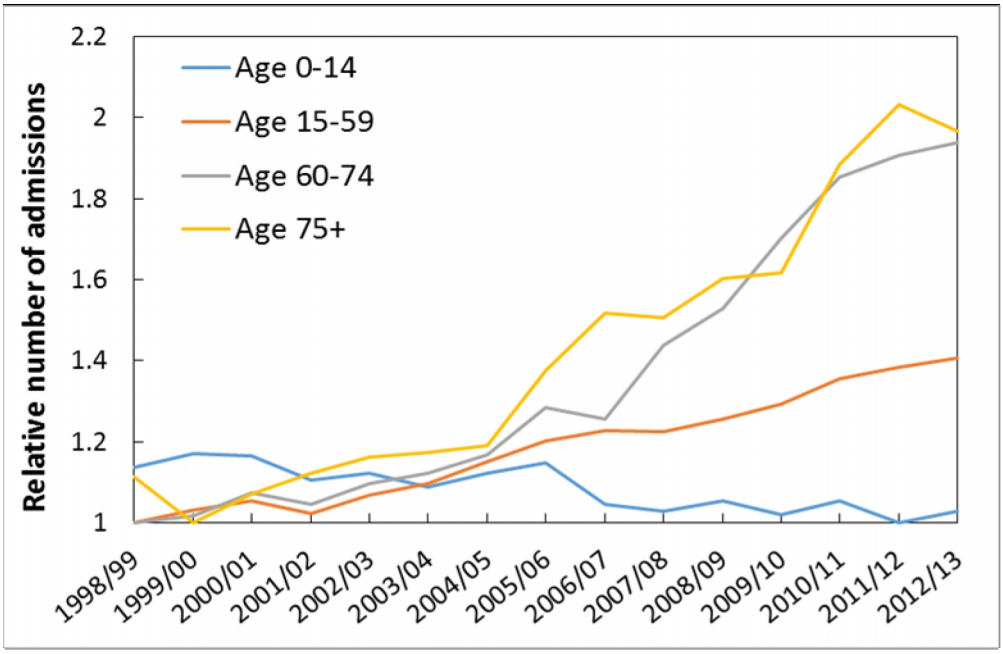

Fig. 2. Relative number of admissions using four broad age bands
Admissions are relative to the year in which the minimum number of admissions occurred

3.3 Effect of Gender

Gender is another important variable in health care trends. Due to the limitations of the standard tabulations available for the HES data only all-age trends can be examined. Since the majority of admissions relate to appendectomies the male/female split for these admissions are presented in Fig. 5, where a set of clear differences can be seen between the two genders. Firstly the trend for male admissions is far less volatile while female admissions are showing accentuated cycle-like trends. The start of each cycle appears to be around 2003/04, 2008/09 and 2012/13 and the significance of these dates will be discussed later.

Secondly, the male trend line appears to be roughly linear with a slope of around 265 additional admissions each year while the female trend line may go through a minimum around 2001. However, the trend after 2003/04 (at the commencement of a cycle) gives around 420 additional admissions per annum which is considerably higher than that for males, such that the two trend lines almost intersect in 2012/13. There is clearly a degree of enhanced female-specificity in the overall trends.

It is of interest to observe that the literature has consistently noted a male predominance, at least in children and young adults [27-28]. However the gender ratio swaps for elderly patients who are more prone to a complicated course and fatal outcome [28] and the female trends observed here are therefore of great significance regarding the escalating rates with age.

Having determined that the trends are largely driven by non-demographic forces and that there are age- and gender-related effects, the final part of this study will focus on attempting to provide an explanation for the observed behavior which is consistent with our knowledge of disease of the appendix, long-term studies conducted in other countries, and recent developments in virology and immunology.

3.4 Diagnostic Uncertainty

As with most other diseases/conditions there is a degree of diagnostic uncertainty relating to diseases of the appendix and even diagnostic imaging is subject to uncertainties [29]. In the US between 1987 and 1998 the incidence of misdiagnosis did not change for children but increased for those aged over 65 years [30]. However in more recent times the incidence of 'normal' appendix declined in Israel from 24% in 1998 to 15% in 2007 [28], and an international rate of 5% to 10% is now commonly encountered [31].

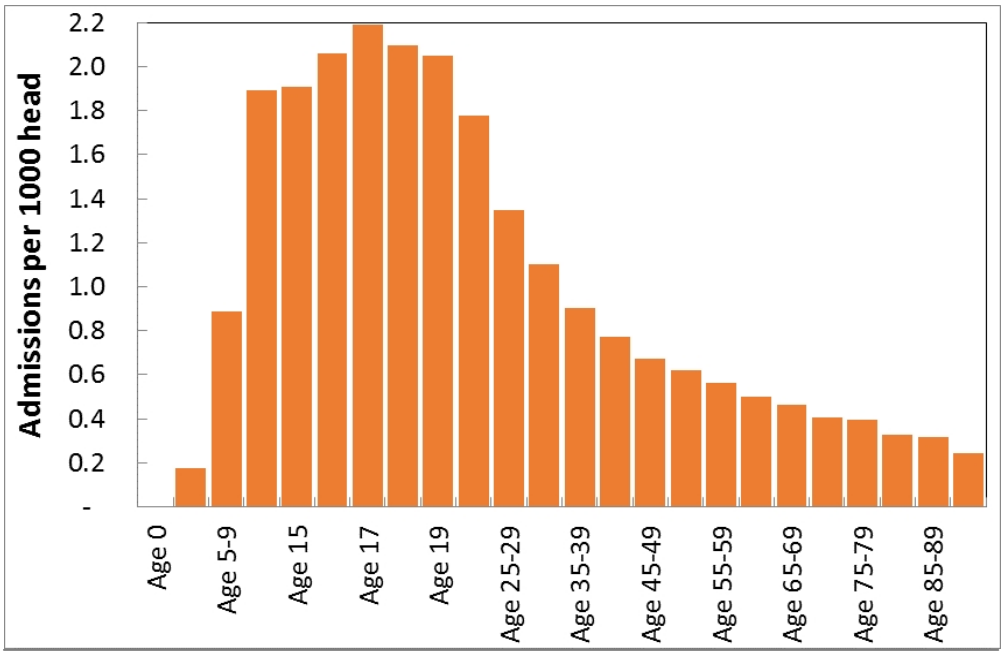

Fig. 3. Admission rates by age band, England 2012/13

Ages 15 to 19 are all single year age bands, final age band is age 90+

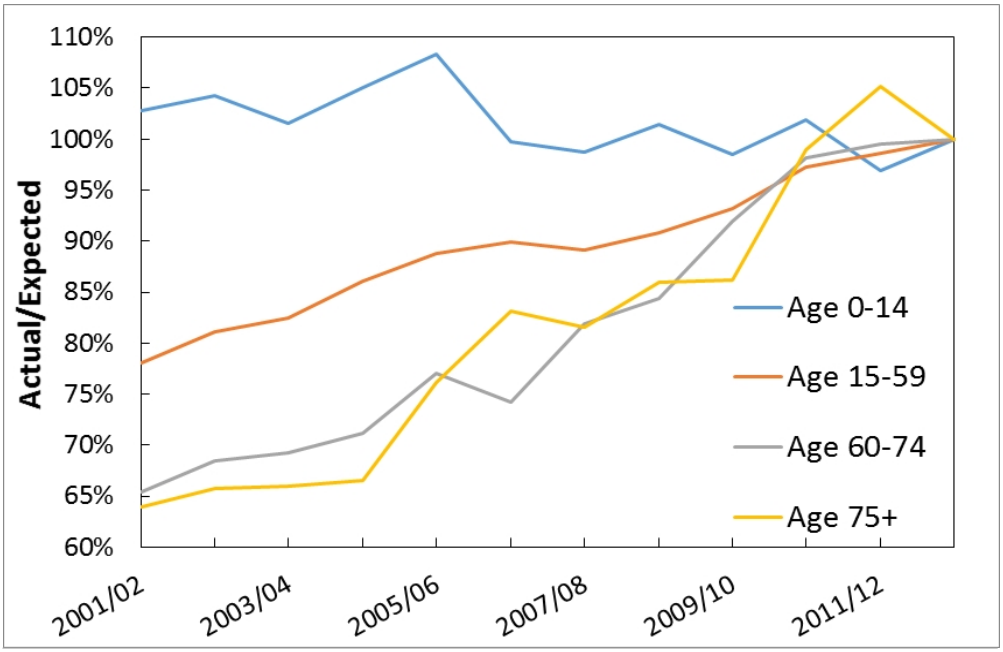

Fig. 4. Actual versus expected admissions in the four age bands over time

Age-specific admission rates in 2012/13 (as per Fig 3) were retrospectively applied to the population of England in earlier years to give expected number of admissions. This was then compared to the actual number of admissions. A steeper slope of the trend- line in this chart represents a far greater contribution from non-demographic based factors

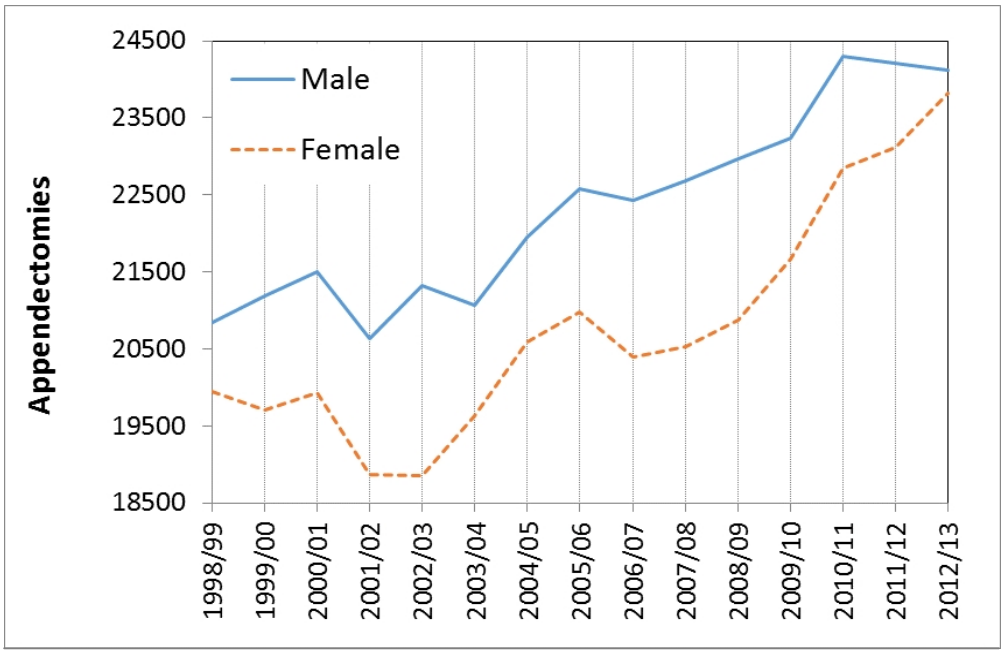

Fig. 5. Trend in male/female appendectomies in England

Inflammatory cancers, perforation and occlusion of the small intestine, peripheral abscesses, fistulae, pylephlebitis and peritoneal inflammatory reactions can be misdiagnosed [29] and complications of Behcet's disease can include an inflamed appendix [32]. Colonization of the appendix by various nematodes, protozoa and other parasites can mimic appendicitis and, depending on country, this affects up to 4% of presenting cases [27]. Diverticular disease of the appendix occurs in around 1% to 2% of cases, although this increases with age and has a 2- to 4-fold higher incidence of perforation [33-34], which makes appendectomy the intervention of choice.

In 'normal' appendicitis there area range of biochemical and inflammatory markers. In those aged over 50 body temperature, heart rate >100bpm, sodium <136 mmol/l, CRP >50mg/l and leukocytes correlated significantly with perforation [35]. An Appendicitis Inflammatory Response (AIR) score incorporating CRP, WBC count, temperature, polymorphonuclear leukocyte proportion and other parameters has been developed to separate negative and phlegmonous appendectomy from advanced appendectomy patients. Additional inflammatory markers such as serum amyloid A, matrix metalloproteinase-9 and myeloperoxidase were also strong discriminators for appendicitis but did not improve the AIR score [36]. A further study noted that high levels of serum bilirubin were more common associated with gangrenous appendicitis [37] while CRP and bilirubin are the most sensitive markers of perforation [38].

There has been increasing recognition that early presentation of uncomplicated acute appendicitis (diagnosed by haematological and radiological investigations) can safely be treated with antibiotics [39]. Use of the AIR score would be beneficial in the selection of patients for antibiotic intervention. However, the potential complicating effects of cytomegalovirus need to be considered and could reduce the efficacy of antibiotic treatment especially in the elderly (see below).

Hence it is highly unlikely that the increasing rates of appendicitis with age observed in this study are due to diagnostic uncertainty, which has reduced over the time interval studied. In this respect, it should be noted that rates of appendicitis in the 0-14 year group (Fig. 3) did show evidence of a small reduction over time. However, from a cost containment perspective this route should be explored for the far larger numbers of younger patients presenting with signs of appendicitis. Somewhere around age 50 may be considered a cut-off given the higher rates of perforation and complication in elderly patients. Using the 2014/15 HRG tariff non-surgical management saves around £1,500 and in England a 10%, 20%, 30% reduction in intervention rates in those aged under 50 would save £5 million, £10 million and £15 million

respectively, i.e. a 5% to 15% reduction in the total cost across all ages and this presents a useful route to mitigating the effects of the age-associated increases in admissions and hence costs.

3.5 Faecoliths in Appendicitis

The topography of the appendicocecal union is reasonably complex and four anatomical types were described by Treves in 1885 [40]. Type I, a smooth funnel shaped cecum with the appendix at the apex occurs mostly in the fetus (100%), first year 80%, up to 10 years 40%, 10-80 years 10% and a lower proportion at older ages. Type III is the most common in adults (increased sacculation of the lateral half of the cecum with the appendiceal union far mesaid to the left toward the ileocecal valve away from the apex of the cecum) in about 79% of cases aged over 20 and may approach 100% above age 80 [40]. The progression away from Type I morphology has a striking resemblance to the increase in incidence with age seen in Fig. 3.

The size of the appendiceal orifice likewise varies from >15 mm in 0.4% of cases through to 0.5 mm in 2.1% of cases with 4-6 mm (33%) and 0.5-2 mm (44%) being the most common [40]. The orifice itself varies from round, oval, irregular, crescent to a slit with prevalence of each varying with size and there are additional mucosal folds (Gerlach's valve) with various forms present in 82% of cases and in adults these folds can fully or partially obscure the orifice in 30% of cases. Given the varied diameter of the opening it is unsurprising that flow rates through the duct vary widely and complete blockage of the duct is sometimes observed. The contractile activity of the appendix also varies and is diminished by infection. This latter observation could support the theory that infection *per se* results in feacolith ingress into the lumen rather than the reverse.

Within this complex anatomical and physiological system the observation of faecoliths (matted fecal material) gained early recognition as a likely cause for appendicitis. The role of faecoliths in appendicitis has been the topic of a recent comprehensive review [15]. The key findings are as follows:

1. Incidence of faecoliths (study conducted in New York, 2001-20110 is markedly different in adults (age >17) and children with prevalence in perforated appendix (adult/child) of 28% and 56% respectively and in non-perforated of 12% and 23% respectively, with perforation only accounting for 11% of adult and 22% of child appendicitis.
2. The incidence of faecoliths appears to have reduced over time
3. It is still unclear as to which comes first, the inflammation or the obstruction
4. Multiple etiologies appear likely and occur with variable frequency in different parts of the world

While a role for dietary fibre has been implicated it is useful to note that a large study over 59 areas in England and Wales concluded that consumption of green vegetables in particular (especially Brussel sprouts and cabbage), some fruits and tomatoes led to a reduction in rates of appendicitis while consumption of potatoes and possibly sugar led to an increase. Rates of appendicitis dropped to a minimum around 200 gram/person/day of vegetables other than potatoes while rates in excess of double the minimum could occur below 140 gram/person/day. Total fibre was not strongly correlated with appendicitis rates [41]. An effect of vegetables on digestive flora was proposed. This link with vegetable consumption probably explains the higher rates of appendicitis and incidence of more complicated disease, in more deprived members of western populations [42-43].

3.6 An Infectious Etiology

3.6.1 The evidence

From the above section it is clear that even if faecoliths were an undisputed cause of appendicitis they cannot explain all appendicitis nor the rising rates of appendicitis with age observed in this study. The evidence for an infectious/immune etiology can be summarized as follows:

1. Depending on age, gender and location presence of fecal matter or foreign body in the lumen only occurs in around 11% to 36% of cases [27,44] and it has never been fully established if this occurs prior to or during appendicitis, i.e. does the fecalith merely act to introduce and then retain infectious material? Of the cases with fecalith 36% are phlegmon, 7% acute, 12% with perforation and 7% with additional findings [44] and fecaliths without appendicitis have been noted. See #2 below.

2. With reference to #1 above, pressure inside the appendix only becomes elevated in late-stage disease, i.e. as a result of inflammation and not as a cause *per se* [13].
3. Lymphoid hyperplasia is present in 13% of cases [27]
4. A variety of bacterial, viral, fungal and parasitic infections have been documented [11].
5. Infectious-like outbreaks of appendicitis have been documented, hence the disease shows clusters in time and location, and differences in incidence between countries and regions [13,45].
6. Persons with sickle cell disease have a 60% lower rate of appendicitis [46].
7. During pregnancy rates of appendicitis is 35% lower during the antepartum period with lowest rate in the third trimester (50% lower) while there is no increased risk post-partum for younger women but is 84% (CI 18% to 186%) higher for those over 35 years [47].
8. In the USA the annual incidence rates of non-perforated appendix and influenza (but not intestinal infection or rotavirus) have shown a parallel U-shaped trend from 1970 to date with a minimum around 1998 [13].
9. The incidence of perforated appendicitis does not correlate with non-perforated or other infections, i.e. it has causative factors which are more complex than simple delay in treating acute appendicitis [13].
10. Incidence of acute appendicitis is slightly higher in the summer than the winter [10,28]
11. In adults, air pollution (via inflammatory effects) is a modifying factor in the incidence of appendicitis, especially in men in the summer months, with highest odds ratio (OR) for the 7-day average values of nitrogen dioxide (OR = 2.4) and carbon monoxide (OR = 1.8) [12]. See #10 above.

The above suggest an infectious/ inflammatory/immune basis for the majority of cases of appendicitis and the apparent relationship with annual incidence of influenza admissions is suggested as a marker of immune impairment rather than actual causation by influenza [13]. These suggest that infectious/ inflammatory forces will determine the trends rather that simple change in the population age structure (demography).

Recent research has indicated a recurring series of seemingly immune-modulating infectious outbreaks, occurring about twice every decade, which have operated in the UK going back to the 1950's [48-54]. The most recent of these outbreaks peaked in the years 2003, 2008 and 2012 although initiation of infectious spread across the UK commenced a year earlier [48,50-55]. The immune modifying ubiquitous herpes virus, cytomegalovirus, has been circumstantially implicated in these outbreaks, either as causative agent (via the introduction of a new strain) or via opportunistic reactivation in response to another agent [49,51,56]. Such an opportunistic association is observed with CMV in HIV/AIDS leading to accelerated decline in health and premature death [57-58].

Cytomegalovirus (CMV) is a common herpes virus. Most people are unaware that they have CMV because, in its mildest form, it causes vague symptoms of lethargy and malaise. In cases in which CMV causes a healthy person to become very sick, the infection may cause the following symptoms and complications (see http://www.mayoclinic.org/diseases-conditions/cmv/basics/complications/con-20029514):

- CMV mononucleosis. This syndrome resembles infectious mononucleosis which is caused by Epstein-Barr virus. Symptoms are a sore throat, swollen glands and tonsils, fatigue, and nausea.
- Intestinal complications. Symptoms are diarrhea, fever and abdominal pain; inflammation of the colon; and blood in the stool.
- Liver complications. CMV can cause abnormal functioning of the liver and an unexplained fever.
- Nervous system complications. A variety of neurological complications have been reported which may include inflammation of the brain (encephalitis).
- Lung complications. CMV can cause inflammation of lung tissue (pneumonitis).

In this respect, it is of interest to note that several studies have demonstrated an association of CMV with appendicitis. In a Greek study involving children (age 6-14) with acute appendicitis CMV was present in 21% and HHV-6 in 8% of cases while HSV-2, VZV and HHV-7 were absent. Of these cases the omentum was infected in 63%, the appendix in 60% and

peripheral blood in 50% of CMV-associated appendicitis [59].

In a Swedish study involving adults (age 16-83) [60], cells from the appendix were double positive for CMV early antigens (IgM) and interleukin's IL-6/IL-8 (markers of inflammation) in 64% of appendicitis cases (60% in phlegmonous (advanced) appendicitis and 67% in gangrenous/perforated appendicitis) and 0% of the control group. CMV early and late antigens (IgM/IgG) were present in 64% and 43% respectively of appendicitis cases but in 0% of the control. While 50% of the control group was CMV positive (CMV DNA in serum) this was elevated to 79% of the appendicitis group. None of the control group had CMV in the appendix [60]. Note that IgM (early antigen) is widely present in the body while IgG (late antigen) is mainly found in the lymph fluid and blood and confers long-term immunity – see next paragraph regarding the role of the lymphatic system. Hence CMV appears associated with appendicitis in between 20% to 60% of cases with the highest proportion reported in adults.

CMV-based appendicitis in those with impaired immune systems has also been observed over many years, such as in HIV/AIDS [61-64], transplant recipients [65], premature infants [66], following an acute Epstein-Barr viral infection [67] and as the presenting manifestation of developing Kawasaki disease [68]. In recently HIV-seroconverted adults there was a 4-times higher incidence of appendicitis, which was higher than average in those not receiving anti-viral therapy, higher viral loads and were younger [69]. The above observation that the omentum and appendix were both infected with CMV [59] is important since both of these organs are part of the wider lymphatic system, and as such, are part of the immune system. Hence both take part in the circulation of white blood cells and antigen presenting cells (dendritic cells) and will therefore be subject to local lymphadenopathy (enlarged lymph nodes) and lymphedema (accumulation of lymphatic fluid) which could well be the ultimate source of appendicitis. CMV is well known for its ability to infect the tissues of the lymphatic system (especially endothelial tissue) and lymphatic organs have been the source of a number of the clinical isolates of this virus which have had the full genome sequenced [70]. A case report of a 38 year old health male with fever and fatigue for two weeks due to CMV reported multiple lymphoid nodules in the bone marrow demonstrating the extent of potential wider lymphatic involvement by CMV [71]. Under favorable conditions CMV doubling time in vivo of just 1 day is observed [72] which would allow rapid development of acute appendicitis.

It is interesting to note that the undulations in hospital admissions over time emanating out of the outbreaks can be discerned in Figs 4 and 5, with a lag of about one year for the 15-59 year age group extending to a lag of around two years for the older age groups. A wide range of medical conditions have been shown to follow this undulating pattern along with variable time lags which could depend on the wider immune-based pro- and ant-inflammatory cascade operating in each disease [73-76].

CMV reactivation is known to occur in an inflammatory microenvironment [60] and at this point it is apposite to highlight the relevance of the study regarding air pollution and a seasonal increase in the incidence of appendicitis where a specific male sensitivity was noted in the summer months [12]. In this respect Fig. 5 shows the ratio of male to female appendectomies over the period 1998/99 to 2012/13. As can be seen the proportion of male appendectomies follows a cyclic pattern relative to each outbreak and appears to become enriched in females as the outbreak progresses (note that the 2012 outbreak actually commences spread across the UK in 2011). A specific effect against females in these outbreaks has been consistently noted [49] and this corresponds with the known differences between the genders in the immune response to CMV, mainly against endothelial cells [77], in vascular disease [78]. In this situation males mount a stronger pro-inflammatory response which is known to be protective against the effects of CMV, especially in the elderly [79]. In this respect, inflammation induced by air pollution will be of a different type to that induced by an infectious outbreak and such differences appear to account for the differential gender responses.

An additional circumstantial finding relates to the 5-fold increase in perforation and abscess for children under the age of 5 years. This age group are subject to a selective deficiency of CD4 T cell immunity toward CMV [80] which is especially so under the age of 2 where there is an undetectable CD4 response to CMV [81]. This potential association requires further research.

A study in the US between 1993 and 2008 (the period when nonperforating appendicitis was increasing) showed a reduction in the 10-19 age

group which confirms the reduction seen for children in this study, however, rates increased in the 30-69 age group. This increase was restricted to Hispanics, Asians and Native Americans while rates in whites and blacks decreased [82]. There have been marked changes in the ethnic composition of England during the time of this study and this potential factor requires further study. Lastly, the apparent association with age in Figs. 1 to 3 also concurs with the known roughly linear increase (in affluent Western countries) in the proportion who are CMV seropositive with age [83-84]; and with the enhancing effects of CMV upon human ageing with associated potentiated effects against the elderly and higher levels of active CMV infection [85-91] and reduced life span [49,92-93].

The role of CMV in cancer and liver disease provides clues to the mode of action of this virus. CMV occurs in far higher frequency in cancerous tissues [94] and in liver disease [95], both of which are immune impaired situations. In cancer, CMV is considered to be oncomodulatory, i.e. hastening the course of the development of the cancer and complicating its treatment [96]. For example, in breast ductal cancer CMV was shown to be present in 97% of cases versus 63% in controls [97], while CMV re-activation has been associated with the onset of breast cancer in others [98].

Hence higher prevalence of CMV in appendicitis is to be expected and it is also expected to hasten its development and create complications in particular cases. CMV is also likely to be more prevalent in appendicitis where the individual has additional immune impairments such as diabetes [99], certain types of autoimmune disease(s), cancer, etc. A link between appendicitis and the subsequent development of colorectal cancer has also been suggested [100]. In this respect, CMV is suspected of being oncogenic in salivary gland ductal cancer [101].

3.6.2 Appendicitis and diverticulitis

It has recently been proposed that nonperforating appendicitis and nonperforating diverticulitis are different manifestations of the same underlying colonic process [102]. In the US both show a U-shaped trend over time with a minimum around 1998 while perforating appendicitis and perforating diverticulitis both show a relatively linear trend over time [102]. Both the perforating and nonperforating trends show high levels of cointegration between US states but no cointegration between the two. Interestingly polymorphisms of the IL-6 gene are associated with nonperforating appendicitis (see Section 3.7.3). The same U-shaped relationship was observed between nonperforating appendicitis and admissions for influenza [13]. In this respect it is of interest to note that an outbreak of appendicitis was associated with an outbreak of an upper respiratory infection [45] and the outbreaks of the disease suggested to lead to undulations in the rate of appendicitis observed in this study has a strong respiratory [51] and digestive system involvement (in preparation).

It has also been noted that appendicitis in the young confers protection against inflammatory bowel disease (IBD) in later life. Appendectomy appears to induce a delayed but significant suppression of genes relating to endothelin activity. Endothelins participate in vasoconstriction and vascular remodeling and endothelin activity is elevated in IBD patients [103]. CMV is well known for its role in the exacerbation of IBD [104] and by inference is likely to be similarly involved in exacerbation of nonperforating appendicitis.

More recent work suggests that appendectomy may also be effective in treatment of ulcerative colitis in adults [105] and in a HIV/AIDS patient initiation of antiretroviral therapy (which can initiate immune reconstruction inflammatory syndrome) appeared to initiate CMV attributable acute appendicitis, which after three weeks was followed by CMV colitis which resolved with antiviral medication [106]. Given the known role of CMV in exacerbation of ulcerative colitis this suggests that in certain cases the appendix could be acting as a CMV reservoir. This is apposite given the strong circumstantial link between increased digestive system deaths and admissions observed to occur during the 2012 outbreak (in preparation). However while appendicitis appears influenced by these outbreaks the link is certainly not as direct as has been observed for respiratory deaths and admissions [51]. The link with respiratory infection is discussed further in the next section.

At this point a case report for confirmed CMV appendicitis may be helpful [107]. An apparently immunocompetent 24 year old Caucasian male with a six year history of primary sclerosing cholangitis and ulcerative colitis was admitted following a 20 day history of fever and upper quadrant abdominal pain. At admission CMV IgG

(11.4 U/ml) and IgM (11.2 U/ml) were considered negative (>20 U/ml) and colonoscopy showed extensive erythema of the colonic mucosa while biopsy showed focal atrophy of the mucosa with edema and chronic inflammatory infiltrates. Imipenem therapy was substituted by tigecycline, however fever persisted. On day eight (28 days after the start of symptoms) CMV DNA was detected in the blood at less than 253 copies/ml but by day 11 this had risen to 6189 copies/ml, with 1431 copies/ml in the urine, and CMV IgG (79 U/ml) and IgM (69 U/ml). Fever rose and pain intensified and appendectomy on day 12 confirmed CMV DNA in the appendix (1210 copies/ml), CMV culture positive and immunohistochemistry positive. It was proposed that the moderate lowering of CD4 T cell count due to the chronic inflammatory state favored CMV disease. However it should be evident that the CMV disease was probably present far before CMV became evident in the blood and such hidden infections have been reported in IBD where the infection can only be discerned by the detection of CD8 effect or T cells (which contain perforin and granzyme B) [104]. Hence the opportunity for considerable under-diagnosis for the role of CMV.

3.6.3 CMV and influenza admissions

The study in the US showing a parallel relationship between hospital admissions for influenza and nonperforating appendicitis [13] is of great relevance regarding the potential role for CMV in such trends. CMV and its associated effects upon certain immunological aspects of ageing is well known for its ability to interfere with the efficacy of influenza vaccination in the elderly [108-110]. The response to influenza vaccination is known to be influenced by factors such as prior treatment for seven months with a complete nutritional supplement [111], serum Vitamin E level [112] and upon the influenza strain [111]. This influenza strain specificity may explain the reported negative impact of CMV in a study using vaccination with the 1997/98 influenza vaccine [113] and upon the stronger effect of CMV upon younger adults with 2009 H1N1 vaccine [114]. Dual infection with CMV and influenza has been shown to lead to a 6-fold increase in the production of IL-6 by endothelial cells leading to vascular inflammation [115]. In children, CMV infection predisposes to common winter respiratory infections [116]. One hospital case study documented a case of necrotizing pneumonia with diffuse alveolar damage and hemorrhage in dual CMV/influenza infection compounded by non-HIV CD4 deficiency [117]. CMV is also involved in potentiating the effect of other pathogens – which is discussed in more detail in section 3.7. An outbreak of the proposed infectious agent (CMV?) has also been proposed to potentiate the effects of influenza and other winter viruses in the winter following the outbreak [118]. Hence the association between influenza *hospitalization*, as a measure of the CMV-enhancing effects upon influenza and appendicitis has a potential common linkage via the immune modulating effects of CMV. In addition, influenza infection is itself an immune weakening event [119-120] which could lead to re-activation of other pathogens which is covered in section 3.7.2.

3.7 Infectious Burden and Multimorbidity

The role of infection with multiple agents, called the infectious burden, is becoming increasingly recognized both as a source for chronic inflammation and the re-activation of latent infections [121]. Due to wide-ranging effects against immune function CMV has been shown to be a key player in this field. Several aspects are of clinical significance, namely, infection with multiple CMV strains, joint infection with CMV and other agents, human genetic variants and differential immune responses between the genders. These will now be discussed.

3.7.1 Infection with multiple CMV strains

The genome of CMV shows remarkable diversity [70,122-125] with a consequent wide variety of strains which show regional differences in prevalence [49,126]. As a result of this diversity CMV exhibits the highest degree of intra-strain sequence variation of any human herpes virus [127]. A family of capsular glycoprotein variants also exist [128-129] which exhibit variable infectious kinetics against different cell types [130] and have different prevalence in organs and patient groups in clinical contexts [127,131]. An Australian study of CMV strains associated with congenital and perinatal infections showed four dominant glycoprotein types with 22 sub-types [128]. There were further longitudinal patterns of infection and the strains were different to those from invasive HIV patients [128].

The pattern of glycosylation in glycoprotein N has been shown to protect CMV against the action of neutralizing antibodies [132]. Mutations in the UL133-UL138 region of the genome regulate the

ability to infect endothelial tissue [133] which is the most common target for CMV infection in a variety of clinical contexts [49,134]. Super infection with multiple strains is common with up to seven glycoprotein variants documented in an individual [135] and the mix and relative proportion of strains in an individual varies over time [122,136] and can depend on the antiviral protocol and the immune response of the patient [137-138]. The course of CMV disease has been noted to be more severe in mixed strain infections [49,124,139]. In the mouse CMV mixed strain infection leads to enhanced viral fitness by functional trans-complementation which occurs at the level of a single cell [140] and competition between strains leads to the complete exclusion of particular strains from particular organs [141]. Also note that CMV-mediated cell death which leads to tissue necrosis (a major factor in appendicitis) is a complex balance between multiple pathways which depend on the specific CMV strain [142]. Hence it has been proposed that the infectious-like outbreaks may be due to the introduction of a new strain of CMV [49] and could therefore be an explanation for the cyclic patterns in appendicitis and gender observed in this study.

3.7.2 Mixed infection with other pathogens

In the intensive care setting CMV is well recognized as a serious pathogen in its own right, however, in the presence of other pathogens these effects are potentiated, with the degree of synergy depending on the organ(s) affected [143].

Several patient case studies document serious multiple infections with CMV and other pathogens such as a patient with severe lymphocyte depletion due to HHV-6 reactivation which then progressed to a fatal dual CMV infection [144], a joint Pneumocystis jiroveci/CMV infection exacerbated by renal failure due to vasculitis (CMV-facilitated?) [145], double encephalitis due to joint HSV/CMV infection [146], a fatal joint Norcardia cyriacigeorgica/CMV infection in a renal transplant patient [147] and fulminant pneumonia due to joint Mycoplasma pneumonia/CMV infection in a young immunocompetent patient [148].

Examples of relevant interactions in mixed infections are as follows: CMV and age are known to interact leading to enhanced HSV-1 reactivation [149], dual EBV/CMV infection leads to enhanced CRP and IL-6 production (a higher inflammatory burden) in those with higher antibody levels to both pathogens [121]; CMV interferes with the host response to the EBV infection [150]; mixed CMV/EBV infections are detected in more than 90% of granulomas in large apical periodontic lesions [151] and in CMV positive cases of Hodgkin's disease EBV was also present in over 85% of cases [152], mixed CMV/hepatitis-C virus (HCV) infection leads to chronic HCV infection with higher liver enzymes [153] and CMV interferes with interferon-based therapy against HCV [154], CMV/Chlamydia trachomatis infection leads to chlamydial persistence [155], CMV/Varicella-zoster virus (VZV) mixed infection leads to enhanced VZV reactivation with age [156], dual CMV/human papovavirus (JCV) leads to JCV expression in cells where this virus is normally non-permissive [157], and there is an association between mixed CMV/Chlamydia pneumonia infection and unstable angina pectoris [158]. In mice, joint infection with Perphyromonas gingivalis/CMV increases both morbidity and mortality, an effect which is not observed with joint *E. coli*/CMV infection [159].

Mixed infection of human organs with herpes viruses is very common and in one study of 8 cadavers across 39 tissue types the following average frequencies were recorded (percentage of infected tissue types): EBV (25.3%), HHV-6 (17.6%), HSV-1 (10.2%), HHV-7 (6.7%), CMV (4.2%), VZV (1.3%), HHV-8 (0.6%) and HSV-2 (0.3%) [160]. Unfortunately the appendix and omentum were not part of this study, however, it is of interest to note that the number of herpes viruses per subject ranged from 3 to 8 with the most common being 3 and only EBV and HHV-6 were present in all subjects A specific role for joint CMV/EBV infection can be discerned in the previous studies and both of these viruses reactivate more frequently in elderly subjects who are co-infected [161] and this synergistic association deserves further study.

In another larger study of 13 common pathogens in Mexican Americans only 0.2% were free from the 13 common pathogens, while the modal value (most common) was infection with 8 pathogens (24% of persons) and 11.3% had 10 or more pathogens. While prevalence to each pathogen generally increases with age the age profiles can show peaks and troughs [21] which could suggest year of birth cohort effects.

This multiplicity of pathogens has not escaped the attention of researchers and cumulative

effects due to the 'pathogen burden' have been noted in atherosclerosis and thrombosis [162], coronary artery disease [163], stroke [164], billary cirrhosis [165] and cognitive function [166]. CMV is a common denominator in these studies.

While the infectious burden is usually determined by antibody tests using the blood it is apposite to determine the level of multiple infection of individual tissues/organs. Data in the above mentioned study of herpes viruses [160] has been summarized in Table 1 which lists all organs/tissues where the DNA from two or more herpes viruses was detected. While this was only a small study of eight cadavers (4 male/female) Table 1 is rich in tissue from the digestive and lymphatic systems. Given that the appendix is part of both these systems this observation is useful in that it points to the potential of a role for infectious burden in initiation and/or exacerbation of appendicitis.

Hence it is almost certain that both the digestive and lymphatic tissues are able to act as viral reservoirs for further infection of other parts of these systems and other organs throughout the body.

While this study has established that the trend for appendicitis in children is not showing any increase it is apposite to look more deeply into the study relating to the presence of herpes viruses in children with appendicitis [59]. Firstly, testing of the appendix, omentum and peripheral blood showed that HSV-2, VZV and HHV-7 were absent in all three locations. This needs to be confirmed in adults. DNA for either CMV, HHV-6, EBV or HSV-1 (with % CMV in brackets) was detected in appendix 61% (11%), omentum 63% (25%) and blood 50% (22%), while CMV was present in at least one location in 74% of patients (versus 54% in the control group), HHV-6 in 47%, EBV in 8% and HSV-1 in 5% [35]. HSV-1 was only ever found in the appendix. CMV and HHV-6 DNA were present in the appendix (11% vs 56%), omentum (25% vs 22%), blood (22% vs 0%), appendix + omentum (7% vs 6%), omentum + blood (7% vs 0%), appendix + omentum + blood (29% vs 16%) [59].

Table 1. Organs and tissues infected by two or more herpes viruses. Adapted from Chen & Hudnall [160]

Tissue	Maximum no of herpes viruses	None detected (%)	Average no of herpes viruses	Number of samples (n)
Parotid gland (salivary)	4	0%	2.3	3
Small intestine	4	13%	1.8	8
Large intestine	4	14%	1.4	7
Nasal mucosa	3	0%	2.0	5
Lymph node (LN) cervical	3	14%	1.7	7
Stomach	3	25%	1.1	8
Urinary bladder	3	63%	0.6	8
Tonsil	2	0%	2.0	3
Submandibular gland (salivary)	2	0%	1.6	5
Lymph node mediastinal/abdominal	2	0%	1.7	3
Spleen	2	13%	1.4	8
Liver	2	13%	1.3	8
Rectum/Anus	2	20%	1.2	5
Tongue	2	25%	1.0	8
Thymus	2	25%	1.0	4
Bone marrow	2	29%	0.9	7
Blood	2	40%	1.0	5
Thyroid gland	2	43%	0.7	7
Dorsal root ganglion	2	50%	1.0	2
Bone	2	60%	0.6	5
Pleura	2	63%	0.5	8
Smooth muscle	2	67%	0.7	3
Vagina	2	67%	0.7	3
Trachea	2	71%	0.4	7
Vena cava	2	71%	0.4	7
Aorta	2	75%	0.4	8

Footnote: n = number of tissues samples. Digestive system organs in blue; lymphatic system in red

All CMV only children presented with typical CMV symptoms (fever, generalized adenopathy, etc) while the HHV-6 only children presented with a generalized rash or adenopathy and fever and the mixed CMV/HHV-6 child presented with generalized adenopathy and retinitis. The fact that there was only one child with a mixed infection appears to argue against a prominent role for infectious burden in children, which is consistent with the proposal that infectious burden increases with age. Given the high proportions of phlegmonous (53%) and perforated (47%) in the study group only a larger study will determine if this represents opportunistic reactivation and how this contributes to disease progression. Comparative rates of appendicitis also need to be established in CMV seropositive versus seronegative children, although allowance needs to be made for the presence of the specific CD4 T cell indifference to CMV in the very young [80-81].

3.7.3 Roles for human gene variants

There are a vast variety of human gene variants which can be researched via the Human Genome Epidemiology (HuGE) navigator (http://www.hugenavigator.net/HuGENavigator/home.do), a searchable knowledge base on gene variants and genetic associations. Genetic variants relevant to CMV infectious potential include an IL-28B nucleotide polymorphism leading to better control of CMV replication [167], roles for co-stimulatory molecule gene polymorphisms in active CMV infection of hematopoetic stem cell transplant recipients [168], Toll-like receptor gene polymorphisms and congenital CMV disease [169], Apolipoprotein E-epsilon 4 genotypes and interactions between the levels of CRP and CMV antibodies [170] and polymorphism in mannose-binding lectin gene and pediatric CMV infections [171]. These are examples of over 100 gene studies relating to CMV infection in various clinical settings. A potential role for IL-6 gene variants can be inferred from section 3.6.2.

3.7.4 Role of gender

While the male and female immune system is nominally composed of the same components they exhibit divergent responses to pathogens and inflammatory stimuli [172-173]. CMV seropositive occurs earlier in women and remains generally higher than men due to seemingly biased transmission of CMV during intercourse [174] and CMV-mediated immune recovery uveitis is more prevalent in females and is seemingly related to a poor CMV-specific response in CD4 T cells [175]. Gender differences are demonstrated in Fig. 5. The proposed infectious outbreaks leading to the cyclic undulations in appendicitis also show gender specificity, especially against females [52]. All future studies in this area therefore need to analyze the male/female response as separate entities.

In summary, co-infection with a range of CMV strains and other pathogens (especially EBV in adults) creates complex disease patterns which arise from differences in the immune responses between the genders and the multiplicity of human genetic variants that constitute each individual. Accumulating pathogens with age therefore contributes to increasing multimorbidity and hence the possibility of enhanced levels of hospitalization for a wide range of conditions (including appendicitis) which increases at a rate greater than simple demographic-based change. This will be especially noticed as the lifespan increments in western populations.

3.8 Limitations and Future Research

This research was limited by the use of standard HES data tabulations which did not allow the decomposition of the trends into age-gender sub-groups. Analysis has been at national level and given the known spatial movement of the recently identified disease outbreaks [48,50,52-53,55] it would be useful to analyze monthly trends in admissions for diseases of the appendix at Local Authority (LA) level. There are around 320 Local/Unitary Authorities in England giving about 160 appendix admissions per annum in each. After splitting these into male/female this is probably too small to perform statistically significant analysis. Realistic analysis will therefore be restricted to locations with more than 500,000 population such as Manchester, County Durham, Bradford, Cornwall, Sheffield, Leeds or Birmingham which would allow a male/female split plus several age bands.

The alternative would be to group LA's according to the date at which the disease outbreak occurred [55] and analyze clusters containing >500,000 population. This approach should be able to answer the question as to whether the changes in rates of appendicitis are directly linked to the outbreaks. A direct link with CMV also needs to be established via a national program of specific screening of the appendix

and omentum for the presence of CMV and other relevant pathogens. Regarding the role of CMV it should be noted that CMV is far more prevalent in deprived populations and this may well be a contributory factor to the well documented association between higher levels of medical admissions and deprivation score [176].

One of the less understood features of CMV infection is the ability to elicit immune suppression even during latent infection. The ongoing immune response during latency is composed of CD4+ T-cells which are specific for viral proteins produced during latency and include T-cells which secrete cIL-10, an immunosuppressive cytokine [177]. This has clear implications to the classic view that CMV must be in a state of active infection before clinical symptoms can arise.

The link between a respiratory acquired infectious etiology and appendicitis requires further investigation as does the apparent link between appendicitis and other diseases of the colon.

4. CONCLUSION

Appendicitis appears to be a condition with multiple etiologies involving obstruction by agents including faecoliths and/or a variety of infectious agents. A likely association between CMV and appendicitis (including other colonic illnesses) has been demonstrated within the context of an age-associated increases in admission rates. CMV appears in some way associated with 20% of cases in children and around 60% of cases in adults. As yet it is not clear to what degree CMV is 'causative of' or 'contributory to' the course and severity of appendicitis. Increasing infectious burden with age is a potential explanation for the increasing admission rates observed in this study and in other conditions. The wider implication to escalating health care costs specific to other medical conditions/ages should be apparent and has been shown to especially apply to admissions for those aged 75+ [19-20].

The recurring series of infectious outbreaks observed in the UK appear to have some effect on the time trends for diseases of the appendix (and wider diseases of the digestive system). The admission avoidance and cost savings achieved via integrated care do not protect against these infectious outbreaks and in Torbay, an integrated care locality, a 7% step-increase in deaths (median increase at Local Authority level across England and Wales was 8.4%, interquartile range 5.8% to 10.9%) occurred in early 2012, as was commonly observed across the UK, i.e. while the cost savings arising from the integration of health and social care are real they do not prevent the cyclic nature of deaths, admissions and cost pressures arising from the outbreaks [26,178].

It would seem that the real problem, for the observed marginal changes in admissions and costs [52,178], may not be the perceived deficiencies within the parts of the health service (relevant in the correct context), but the inability/unwillingness of the authorities to recognize the existence of a recurring series of powerful infectious events with profound public health importance. These outbreaks are then creating a unique set of financial pressures and resource constraints (of which increasing rates of appendicitis is but one example). Cause and effect must be correctly attributed to avoid trivializing the important, inappropriate forecasting of future activity based on simplistic demographic-based tools and unrealistic assumptions regarding the long-term efficacy of proposed solutions.

CONSENT AND ETHICAL APPROVAL

Not required, analysis of publically available data.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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