A dynamic transmission model with age-dependent infectiousness and reactivation for cytomegalovirus in the United States: Potential impact of vaccination strategies on congenital infection

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We present an age-structured dynamic transmission model for cytomegalovirus (CMV) in the United States, based on natural history and available data, primarily aiming to combine the available qualitative and quantitative knowledge toward more complex modeling frameworks to better reflect the underlying biology and epidemiology of the CMV infection. The model structure explicitly accounts for primary infections, reactivations and re-infections. Duration of infectiousness and likelihood of reactivation were both assumed to be age-dependent, and natural reduction in the re-infection risk following primary infection was included. We used an empirical social contact matrix (POLYMOD-based) as support for CMV transmission between different age groups. The baseline model reproduced well the age-stratified seroprevalence data (National Health and Nutrition Examination Survey III) used for calibration. The model was further used to explore the potential impact of hypothetical vaccination on reducing congenital CMV infection under various vaccine profiles and vaccination scenarios. Our preliminary model-based simulations suggested that while infant vaccination may represent an attractive way to reduce congenital CMV infection over time, adolescent female vaccination with an adequate routine booster platform may, under certain conditions, provide an alternative. However, for such tools to be considered toward actual decision-making, enhanced validations based on additional studies and data would be further necessary. The modeling framework presented in this paper was designed to be sufficiently general and flexible, such that it can allow for further adaptations to reflect new knowledge or data that may become available in the future.

Introduction

Human cytomegalovirus (CMV) is an ubiquitous β-herpes virus that causes mostly mild and asymptomatic infections in immunocompetent individuals, but can cause serious disease in fetuses and immunocompromised individuals (e.g. HIV-infected individuals, organ and stem cell transplant recipients).1 Its natural history is characterized by a primary CMV infection followed by a life-long latent infection that can reactivate periodically resulting in shedding of infectious viral particles.2 Re-infection with a different CMV strain can also occur, with considerable evidence suggesting that immunity resulting from initial infection may provide some level of protection against re-infection.3 CMV is transmitted through contact with CMV-infected body fluids (urine, blood, saliva, genital secretions) of individuals with symptomatic or asymptomatic CMV infection, via close non-sexual contact, sexual contact, breastfeeding, blood transfusion, or organ transplantation.4,5 CMV infection may also be transmitted from mother to fetus, congenital CMV (cCMV)
infection being the most common intrauterine infection with severe sequelae, such as deafness, neurological disabilities and visual impairment.\textsuperscript{6} Congenital CMV infection results from \textit{in utero} transmission from the mother to her fetus, either because she undergoes a primary CMV infection during pregnancy or because she experiences a recurrent CMV infection, defined as either viral reactivation or re-infection with a different strain of CMV during pregnancy.\textsuperscript{7} A fetus is at highest risk of contracting CMV infection when the mother has a primary infection during pregnancy; the risk is substantially lower for fetuses with mothers experiencing recurrent infections while pregnant.\textsuperscript{1} However, a large proportion of congenital CMV infections can be attributed to non-primary infections in mothers.\textsuperscript{8} In the United States (US), 30% to 50% of women of childbearing age have never been infected with CMV; 1% to 4% of uninfected women experience a primary CMV infection during a pregnancy and in about 33% of these cases the virus is transmitted to the fetus.\textsuperscript{9}

In the US, CMV is the most common viral infection diagnosed at birth: about 1 in 150 newborns has a congenital CMV infection, while 1 in 750 newborns has or develops permanent sequelae due to cCMV infection.\textsuperscript{9} Hence, every year, of the approximately 28000 children born with congenital CMV infection, more than 5500 suffer permanent disabilities.\textsuperscript{10} Congenital CMV infection in the US causes more long-term problems and childhood deaths than the Down syndrome, the fetal alcohol syndrome, or neural tube defects.\textsuperscript{11,12} Overall it has been estimated that 17–20% of the newborns with symptomatic and asymptomatic cCMV infection, at birth, develop permanent sequelae.\textsuperscript{4} About 13% of newborns diagnosed with cCMV infection are symptomatic, with rates of permanent sequelae ranging from 40% to >80% according to the severity of their disease. Intrauterine growth retardation, prematurity, hepato- and splenomegaly, petechiae, microcephaly, neurological deficits, seizures, or chorioretinitis are few of the sequelae observed in newborns with symptomatic cCMV infections.\textsuperscript{1,13,14} In case of asymptomatic cCMV infected children, sensorineural hearing loss was observed in 7.4–21% of children 3–4 y old, but late-onset hearing loss after the age of 7 y was also reported.\textsuperscript{15,16} Therefore, congenital CMV infection and disease are primary targets for prevention, and there is scientific evidence suggesting that viral transmission can be reduced by limiting the risk of infection through hygiene measures or vaccination.\textsuperscript{17,18} Hence, a better understanding of CMV transmission is important to evaluate potential impact of preventative measures, such as vaccination, at population-level.

In 1999, the Institute of Medicine has included the development of a CMV vaccine among the high priority targets, thus recognizing its potential implications for public health in terms of reducing the economic costs and human morbidity.\textsuperscript{19} Despite significant progress in virology, immunology, epidemiology, and the availability of several candidate vaccines in early development stages, no product is yet under consideration for licensure.\textsuperscript{20,21} There is a need for vaccines able to prevent primary infection with CMV and capable of boosting immune responses in already infected individuals.\textsuperscript{19} Although a CMV vaccine would mainly target adolescents and women of childbearing age, young children could also be targeted since vaccination in this age group may decrease transmission to adults. In addition, the vaccine might be beneficial for patients with AIDS or prospective recipients of transplants.\textsuperscript{2}

Clinical trials of CMV vaccines in women should evaluate protection against cCMV infection, an essential precursor of cCMV disease.\textsuperscript{19} Additional research should include determination of age-specific incidence and transmission rates, description of the processes involved and relative contributions of maternal reactivation as well as re-infection with cCMV disease, developing assays that can distinguish between reactivation and re-infection.\textsuperscript{19}

Dynamic transmission models are valuable mathematical tools to simulate the transmission and spread of infections in a population and to assess the potential impact of preventative measures.

A dynamic transmission modeling framework enables the integration of multiple natural history infection data with relevant biological/medical quantitative and qualitative information to create a unifying working platform. The resulting validated model can then be employed to project at population level the impact of various interventions, including vaccination, using different working assumptions.

To date, limited work on CMV transmission modeling has been published.\textsuperscript{22-24} In general, a key rationale for developing mathematical models is to help bridge what is observed with what is not yet known, and to generate and test hypotheses. The work presented here is intended as a step toward more advanced modeling frameworks that could more realistically reflect the biology and epidemiology of CMV infection, based on qualitative knowledge combined with available data. To this end, we developed an epidemiologically realistic, age-structured, dynamic transmission model for CMV in the US, based on natural history and available epidemiological data, and explored a few different hypothetical vaccine profiles and vaccination schemes and their potential impact on reducing congenital CMV infection.

### Results

**Baseline model**

The best-fit model against the age-stratified National Health and Nutrition Examination Survey III (NHANES III) CMV seroprevalence data for the US population\textsuperscript{25} is illustrated in Figure 1A. The corresponding model-estimated force of infection (FOI: per susceptible risk of infection per year) as a function of age is shown in Figure 1B. The model succeeded in capturing very well the NHANES III CMV age-specific seroprevalence, with a p-value for model-data of 7\times10\textsuperscript{-7} (correlation coefficient value 0.97).

The underlying age-dependent average duration of infectiousness, reactivation rate, and transmission matrix yielded by model calibration under the current model working hypotheses are all fully illustrated in **Supplemental File 1**. The model-estimated value for the natural reduction $\kappa$ in the risk of an individual to acquire a new infection following primary infection was around 65%. The 0.67% target birth prevalence of congenital CMV
infections here was successfully recovered by the model with estimated mother-to-newborn transmission rates of 33.4% and 8.5% following primary versus recurrent maternal infection, respectively. Based on the present model outcomes for the best-fit shown in Figure 1A, an estimated 1.2% of seronegative pregnant women would experience a primary infection, while the corresponding model-projected percentage of seropositive mothers with CMV-positive newborns was 1.1%.

The model-projected percentage of congenital CMV infections attributable to primary infection in mothers was estimated at 29% vs. the percentage of congenital CMV infections attributable to recurrent infection in mothers, estimated at 71% (Fig. 1A). This is in-line with the corresponding estimates of 25% vs. 75%, respectively, published by Cannon.11

The model-based force of infection shown in Figure 1B appears highest in very young individuals, with the dominant peak occurring between 0–2 y of age. A secondary peak is visible between 20–40 y of age, with a local maximum around the age of 32 y, likely reflecting the child-bearing years coupled with the associated risks of acquiring infection from young children.26 The model-based force of infection in the pooled 12–49 year-old age group was estimated at 2% per year, in-line with the corresponding estimate from Colugnati et al.,2 who estimated an average age of infection at 28.6 y.

Model with vaccination

This modeling framework enables consideration of many different vaccination scenarios. The present work was not intended as exhaustive or final, but as a starting point for illustrative purposes. Thus, for the time being, 8 hypothetical vaccination scenarios we considered a priori of primary interest in the context were included in the analysis on top of the baseline model: vaccination of seronegative or seronegative and seropositive female adolescents with/without booster and with/without catch-up strategies; or vaccination of infants <1 y of age irrespective of their CMV serological status with/without booster and with/
without catch-up. Routine vaccination in our model is considered at 6 months and 10 y of age for infants and adolescent females, respectively.

In the present study, similar vaccine coverage and efficacy were considered for routine primary, catch-up and routine booster vaccination. We worked under the plausible assumption that if a platform for boosting later in life is in place, then it would be equally available and offered to both previously vaccinated individuals in whom vaccine protection has waned as well as unvaccinated individuals of the right age. Appropriate set-up of routine booster programs in practice can have an impact by extending protection in those previously vaccinated as well as providing immunity to those previously unvaccinated. Throughout our simulations here, for booster in seronegative women, if the mean duration of vaccine protection was assumed to be 10 y, then routine booster administration applied to seronegative females at 20 y of age (upon vaccine protection waning). If the mean duration of vaccine protection was assumed 20 y, then routine booster applied to seronegative women at 30 y of age (upon vaccine protection waning). In the case of infant vaccination with booster, a routine booster was set at 10 y of age.

In the present simulations, we considered vaccine coverage of 30% (conservative case) and 70% (best case). Target vaccine efficacy was considered 70% (desirable) and 90% (ideal case), with vaccine-induced duration of protection of 10 and 20 y. Our main interest was to assess reduction of congenital CMV infection in each vaccination scenario under the different combinations of these assumed target vaccine characteristics (coverage, efficacy and duration of protection).

Of note, all the hypothetical vaccination scenarios presented here were defined a priori, at the start of the actual analyses and before running any model simulations. In particular, the age of 10 y for routine adolescent vaccination was selected a priori of interest as a potential early age that could be aligned with the existing HPV vaccination platform in the US, where 2 HPV vaccines (Gardasil™ and Cervarix™) are currently in-use for females starting at 9 y of age, given in 3 doses over 6 months, with current routine vaccination recommended by the Advisory Committee on Immunization Practices around the age of 11–12 y. The vaccine coverage rates considered a priori of potential interest here were also based on prior experiences with the HPV vaccine implementation globally. A vaccine efficacy of 70% was also considered in ref., 23 while the selected 90% value was a priori defined as an ideal case for comparison purposes.

The most relevant results are shown in Figures 2–5, illustrating the model-projected reduction in congenital CMV infection birth prevalence over time post-vaccination under different vaccination scenarios. The magnitude of reduction clearly will depend on specific vaccine characteristics: efficacy, coverage, duration of protection, and the various possible subsequent combinations.

The outcomes in Figure 2 correspond to scenario 1 in Table 1: seronegative adolescent female vaccination without booster, with a one-time catch-up in 10–17 y old at start-up, in the base-case vaccination assuming no vaccine impact on reactivation. This is a worst-case scenario in terms of reducing congenital CMV infection, where even in the best case (case 3), with 70% vaccine coverage, 90% vaccine efficacy and 20 y duration of protection, only a reduction of about 5%, 15% and 18% in congenital CMV infection from baseline pre-vaccination could be achieved.
after 10, 20 and 50 y post-vaccination, respectively. Vaccination impact is mainly driven by direct protection effects in this case. Vaccine duration of protection is a key factor here for adolescent women vaccination, as longer durations of protection can extend protection against infection into the child-bearing age, critical for subsequently reducing congenital CMV infection. This could be achieved in practice by appropriate booster platforms later in life.

The importance of a booster platform is illustrated in Figure 3, corresponding to scenario 3 in Table 1: seronegative adolescent female vaccination with booster and a one-time catch-up in 10–17 y old at start-up, in the base-case vaccination assuming no vaccine impact on reactivation. In our simulations here, routine booster at the appropriate age is not only being administered to previously vaccinated women with waned immunity, but also equally to those who have not been previously vaccinated. In the best case (case 3), with 70% vaccine coverage, 90% vaccine efficacy and 20 y duration of protection, with a one-time catch-up in 10–17 y old at start-up and routine booster later in life, the reduction in congenital CMV infection from baseline pre-vaccination could go up to about 38% 50 y after vaccination, compared to only 18% before in the same case but without a routine booster platform, also highlighting that the impact of implementing a booster platform later in life for female vaccination would only become more apparent in the longer term.

If the vaccine was assumed to have no impact on reactivation, vaccinating seropositive in addition to seronegative adolescent females did not add notable benefits for overall reduction of congenital CMV infection. However, in an optimistic vaccination framework, the outcomes can be significantly improved if the vaccine had a similar efficacy on both infection acquisition and reactivation, acting by reducing both the risk of infection and the likelihood of reactivation in vaccinated protected individuals.

Such outcomes are illustrated in Figure 4 corresponding to scenario 2 in Table 1: seronegative and seropositive adolescent female vaccination without booster and a one-time catch-up in 10–17 y old at start-up, in the optimistic vaccination framework, assuming equal vaccine impact on both infection acquisition and reactivation. In the best case (case 3) here, with 70% vaccine coverage, 90% vaccine efficacy and 20 y duration of protection, a reduction of about 15%, 32% and 36% in congenital CMV infection from baseline pre-vaccination could be achieved 10, 20 and 50 y, respectively, after vaccination. With the addition of an appropriate routine booster platform, as before, the reduction in congenital CMV infection 50 y after vaccination could go up to about 70%, as illustrated in Figure 5.

Clearly, Figure 5 illustrates the outcomes for congenital CMV infection reduction in a best-case vaccination framework for adolescent female vaccination, while by contrast Figure 2 depicts a worst-case vaccination framework for adolescent female vaccination.

The variability in outcomes between these 2 extremes here is considerable.

By contrast, the model-based projections in the case of infant vaccination under scenario 5 in Table 1, in the base-case vaccination assuming no vaccine impact on reactivation, indicate that routine infant vaccination without booster or catch-up may provide a reduction in congenital CMV infection of about 18%, 30% and 68% from baseline (pre-vaccination) at 10, 20, and 50 y post-vaccination, respectively, in the worst case (30% vaccine
coverage, 70% vaccine efficacy and 10 y duration of protection, e.g., case 6 in Fig. 6), while in the best case (70% vaccine coverage, 90% vaccine efficacy and 20 y duration of protection, e.g., case 3 in Fig. 6) 33%, 47%, and 97% reduction from baseline (pre-vaccination) at 10, 20, and 50 y post-vaccination, respectively, could be achieved. Duration of protection in this case is less critical than above in the adolescent female vaccination framework, provided it is long enough for the young children to remain protected for a critical time-window (e.g., up to 5 y of age). Adding a booster dose at 10 y of age or a catch-up campaign in this case did not make any further significant impact; neither did vaccine impact on reactivation (results not shown here). Elimination could in theory be achieved at 30% vaccine coverage, 90% vaccine efficacy and 10 y vaccine duration of protection, for instance, but it would take well beyond 100 y post-vaccination. This however corresponds to an ideal case where there would be no importation of the virus; in more realistic simulations, if importation of the virus is mimicked for instance via a small percentage in the force of infection not directly caused by person-to-person transmission (results not shown here), then elimination is no longer possible.

**Discussion**

We proposed and tested a complex dynamic transmission model for CMV in the US population, where we primarily aimed to combine the available qualitative and quantitative knowledge toward more complex modeling frameworks to better reflect the underlying biology and epidemiology of the CMV infection. A few other valuable mathematical models to explore potential impact of vaccination at population-level and rationalize implementation of potential vaccination strategies exist. Our modeling framework has added significant complexity in a first attempt to integrate additional desirable features into a CMV dynamic modeling framework. Novel features include:

- fine age structure and realistic demography
- gender stratification
- distinction between primary infections, reactivations and re-infections, with differential impact on congenital CMV
- age-dependent infectiousness and reactivation
- transmission estimates based on empirical social contact patterns
- model calibration framed as a sophisticated constrained optimization problem, based on the currently available relevant information further translated into corresponding constraints to enforce epidemiological plausibility
- methodology not restricted to steady-state and can be applied in the presence of longitudinal data as well
- versatility, allowing for potential consideration of different vaccine modes-of-action (e.g., reduced susceptibility, reduced infectiousness, reduced risk of mother-to-fetus transmission).

The modeling framework presented in this paper was designed to be sufficiently general and flexible, such that it can be adapted to different settings and data-sets, and also readily allow for further changes to reflect new knowledge that may become available in the future. We do not regard this work as exhaustive or final, but rather as a starting point toward challenging and
advancing the status quo in the field, highlighting the need for more studies and data necessary to bring such modeling tools closer to robust quantitative validations and subsequent use to help inform decision making.

The calibrated baseline model successfully reproduced the NHANES III CMV age-stratified seroprevalence data, while simultaneously aligning with other reports.\(^1\)\(^,\)\(^2\)\(^,\)\(^11\)\(^,\)\(^22\)-\(^24\) For simplicity, and in the absence of compelling data indicating otherwise, the baseline model was calibrated at steady-state, under an assumption of an endemic equilibrium of the prevalence of CMV infection in the US population, supported by the limited available data.\(^10\)

The parameter estimation problem related to the baseline model calibration (e.g., in the absence of vaccination) was framed here as a fairly constrained optimization problem, where qualitative and quantitative available information was translated in constraints, in order to reduce the number of possible combinations. Other dynamic CMV transmission models at population level have worked with simpler assumptions of constant average duration of infectiousness and reactivation rate. In ref. 22 the authors used different values for the average duration of infectiousness, including a 6-month duration (in-line with other published reports e.g. ref. 27) that was subsequently employed in a similar manner in an adaptation of the model by the Centers for Disease Control and Prevention in the US.\(^23\) The 6-month duration of infectiousness was also the estimate that yielded the best fit against the seroprevalence data for our baseline model in the older age groups. Regarding the reactivation rate, the assumption of an S-shaped function characterized by a gradual increase from low levels at young ages to higher levels at old age appeared more biologically realistic to us than just a constant rate across all age groups. This assumption is based on qualitative information that seem to indicate that aging of the immune system may correlate with a progressive decreased ability to properly control CMV reactivation over time, a biological process often referred to as immunesenescence.\(^28\)-\(^30\) A constant reactivation is a simpler assumption that has been considered in other models.\(^22\)-\(^24\) In either case, to our knowledge, there is no direct data that could be readily and robustly used for actual quantitative estimates, which still need to be found via calibration.

As explained in our Methods section, POLYMOD social contact data for physical contacts longer than 15 minutes was employed in this modeling framework solely as a plausible empirical support for transmission for CMV in the US population, under the assumption that the social contact patterns are not very different in developed countries and in EU vs. US. For actual infection transmission, we introduced a correction factor, which is a bivariate parametric function, on top of the social contacts. In the Methods section, we provided a justification for the choice that in this instance led to the best fit against the age-stratified seroprevalence data, but in reality, it is not always easy to attach an appropriate biological rationale to the mathematical expression of this correction factor, and its definition is not obvious a priori. The final form we eventually converged to here, given in the Methods section, was basically reached via an educated trial-and-error process. In a related simpler model in ref., 22 the authors actually proposed an ad-hoc bivariate parametric function for the transmission matrix for CMV, which was calibrated based on seroprevalence data from Brazil, also yielding a bimodal shape of the force of infection with respect to age, dominated by

![Figure 5. Model-projected congenital CMV infection birth prevalence over time post-vaccination of both seronegative and seropositive adolescent females with booster, one-time catch-up in 10–17 y old at start-up, under scenario 4 in the optimistic vaccination framework (with vaccine impact on reactivation).](image-url)
a sharp peak in the youngest and followed by a secondary peak later in life.

The profile of the model-based force of infection as a function of age displays some qualitative trends similar to those previously reported in ref. 22. However, in our model projections, the magnitudes of the primary and secondary peaks are considerably closer, with the primary peak in the very young individuals only about 1.5 times higher than the secondary peak occurring later in life. These differences between the age-specific profiles of the force of infection between our model and those reported by the authors in ref. 22 are reflective of the differences in the age-specific CMV seroprevalence data from US and Brazil, respectively. In the US, the seroprevalence data shows an almost linear increase with age, while the seroprevalence data from Brazil exhibits a more classical sigmoid shape, with a rapid increase in early childhood followed by plateauing around the age of 30 years.22,31 As a consequence, the average age of infection is likely to be considerably lower in Brazil (estimated at 19.84 years in ref. 31, based on seroprevalence data for ages 10–40 years) compared to US (estimated at 28.6 years in ref. 2 based on seroprevalence data for ages 12–49 years). Intuitively, the secondary peak in our current US model corresponds to an enhanced risk of infection in a population most likely to have close physical contact with young children. Fertility rates in the US are about 3 times higher in the 25–30 years old than in the 15–19 years old.32 In addition, at calibration, we did not only fit the age-stratified seroprevalence data in ref. 25 but also the CDC-
reported birth prevalence of congenital CMV infection, along with the estimated value provided in the literature for the force of infection in the 12–49 y old with a number of appropriate constraints as described in the Methods section under Model calibration and validation. The third peak projected by our model here in the older ages is likely a reflection of the still increasing seroprevalence in old age groups, as indicated by the age-stratified seroprevalence data, combined with the assumed empirical contact pattern. Removing the third peak or constraining it to be flatter leads to less good fits against the seroprevalence data in the old age groups; in other words, in order for the seroprevalence curve to keep going up in old age groups, as indicated by the data, something needs to drive it, which in practice may be a consequence of likelihood to have and interact with young grandchildren, for instance. In the present context, however, where our focus is on congenital CMV infection, such potential peaks in the older age groups have little practical impact, as transmission from this segment or likelihood of first-generation offspring are low. We would like to also note here that the force of infection shown in Figure 1B is estimated based on a fine age structure, as enabled by the model, and thus the usual smoothing that naturally occurs in practice by pooling larger age groups is not present here.

To our knowledge, no gold standards currently exist for the shape of the force of infection as a function of age. Traditionally, under the assumption of steady-state, simpler catalytic models can be employed to render a profile for the force of infection with respect to age so that the age-stratified seroprevalence data is matched. However, such approaches often imply using predefined mathematical expressions involving a number of unknown parameters that are estimated by fitting the available seroprevalence data. This is closer to a curve-fitting exercise, with limited epidemiological substance, and different assumed profiles may lead to similar fits against the seroprevalence data. In our case, in the absence of strong evidence, no prior assumption was made about the shape of the force of infection, and the resulting profile is a model outcome based on all the complex underlying epidemiological information included in the modeling framework. This approach is not methodologically restricted to steady-state assumptions, and can well be employed in the presence of longitudinal seroprevalence data as well. If strong evidence becomes available that could be used to enforce more plausible or expected profiles of the force of infection as a function of age, then this can and should be added in the list of constraints for model calibration, and model re-calibrated accordingly. This would naturally impact subsequent model projections for vaccination.

Regarding the model with vaccination, our objective was to use a more realistic calibrated model toward assessing the potential impact of vaccination on reducing congenital CMV infection at population-level. There are many various vaccination scenarios that could be simulated in this modeling framework; for our main goals for the time being, however, the scope was restricted to some illustrative analyses that were a priori considered of primary interest in the context. We want to further reinforce the fact that our aim here was not to find critical threshold values for vaccine efficacy or coverage or age at vaccination that may impact cost-effectiveness analyses and subsequent decision making. Cost-effectiveness analyses and selection of cost-efficient strategies, etc. were out of the scope of the current work. While epidemiological modeling frameworks need to be challenged and evolve toward more realistic ones, this evolution needs to be accompanied by additional studies and data needed further for more robust model assessment and validation to facilitate the appropriate choice of model for actual decision making.

In line with other reports, our preliminary simulations presented here indicated that infant vaccination may lead to a more efficient reduction in congenital CMV infection over time compared with adolescent female vaccination. The model projected a reduction of congenital CMV infection at 50 y post-vaccination in the case of routine vaccination of all infants 6 months of age, irrespective of their serological serostatus and without any catch-up or booster dose (Fig. 6), ranging from 68% to 97% across the 8 combinations of vaccine coverage, efficacy and duration of protection considered. By comparison, in an optimistic vaccination framework assuming equal vaccine impact on infection acquisition and reactivation, routine adolescent vaccination of both seropositive and seronegative females at the age of 10 y without booster, with a one-time catch-up in 10–17 y old at start-up, (Fig. 4), may lead to congenital CMV infection reductions from baseline (pre-vaccination) of 4% to 36% at 50 y post-vaccination. In the same optimistic vaccination framework, adding on top of routine female adolescent vaccination an adequate routine booster platform for vaccination of both seronegative and seropositive females later in life (with a one-time catch-up in 10–17 y old at start-up) (Fig. 5) may lead to congenital CMV infection reductions from baseline (pre-vaccination) of 15% to 70% at 50 y post-vaccination, depending on the combinations of vaccine coverage, efficacy and duration of protection considered. In an ideal case scenario, results for adolescent female vaccination here could be further improved in the event that newborns of vaccinated and protected mothers may also be temporarily protected against infection (not presented).

For all practical purposes, while model-based projections were shown here up to 100 y post-vaccination for a more complete picture, in reality it is highly likely that the validity of such projections decreases significantly with time, as other natural epidemiological changes are likely to occur over time, which are not considered here.

In our present modeling framework, due to the underlying transmission matrix combined with longer duration of infectiousness in children younger than 5 y of age, notable herd protection effects could only be achieved if there is some level of vaccine-induced protection in this particular age group. Even at relatively low effective coverage (e.g., around 30%) of vaccination in infants, for instance, subsequent impact on transmission and herd protection can be significant, with impact on congenital CMV infection reduction already apparent at 10 y post-vaccination. By contrast, such effects cannot be achieved in our current modeling framework solely via adolescent female vaccination, where impact of vaccination on congenital CMV infection reduction appears mostly driven by direct protection effects in vaccinated women of child-bearing age.
However, if vaccinating young children may not be feasible, the alternative could remain for adolescent female vaccination with booster, where aspects like vaccine impact on reactivation may play an important role.

Model-based simulations in ref. 22 indicated that the best strategy in preventing congenital CMV infection in Brazil may be universal CMV vaccination before the age of 12 months followed by a booster dose at 10–11 years, assuming duration of protection of at least 20 y. However, the same model also suggested that in the Brazilian setting, infant vaccination without booster could potentially increase congenital CMV infection if vaccine duration of protection is lower than 20 y. Preliminary analysis in refs. 23, 24 based on the previous Brazilian modeling framework from ref. 22 and adapted to the US data suggested that such a scenario would not occur in the US settings, even at lower duration of vaccine protection (e.g. 5 y or less), and argued that shorter duration of vaccine protection may in some cases have some adverse effects on congenital CMV infection in high CMV seroprevalence settings such as Brazil. In our model-based simulations, we did not find evidences supporting a potential negative impact of vaccination on congenital CMV infections in the US setting in the vaccine scenarios considered.

The vaccine duration of protection is a key factor that can have a big impact on subsequent model projections, particularly in the adolescent female vaccination framework, and we have to acknowledge that the values considered in our analyses here, of 10 and 20 y duration of vaccine-induced immunity respectively, may well be overly optimistic. However, based on the HPV vaccine experience, where long term (>6 y) immunity has been demonstrated,36 we considered the 10 y duration of protection a desirable value, albeit optimistic. For practical purposes, this could be achieved with 5 y duration of protection followed by routine booster for an additional 5 y duration of protection, for instance. The 20 y duration of protection here represents an idealized case, for comparison purposes.

Given the high dimensionality and nonlinear dynamic nature of the problem, different sensitivity analyses can be envisioned in theory, resulting in complex and computationally-intensive exercises. In order to retain value and maintain tractability, they need to be systematic and well defined in scope; to that end, further studies are necessary to collect and collate such data in order to ascertain actual ranges and variability for the different input parameters so as to guide sensitivity analyses. For the baseline model here, we performed a constrained optimization in a parameter space, with the forward problem governed by a fully deterministic model, and we found a set of coupled model parameters that led to the best fit against all available quantitative data. This procedure implicitly means we have searched through the parameter space and ultimately chose an optimal set. There is, clearly, the typical issue that solution of this optimization problem may not be unique, and multiple sets of model parameters (different combinations) may lead to equivalent best fits against the data. We have run the optimization exercise starting from different initial points in the parameter space, with different algorithms (simplex, gradient-based, etc.), and retrieved equivalent solutions in terms of overall system behavior.

In general, for nonlinear and complex models, univariate sensitivity analysis is insufficient for a comprehensive study of the model.37 Multivariate sensitivity analysis is more appropriate, based on the inherent coupling and nonlinear relationships in the model. When parameter values have large uncertainties or models are nonlinear, global sensitivity analysis techniques are more appropriate.38 Global sensitivity analysis techniques investigate the effects of simultaneous parameter variations over large but finite ranges and allow the effects of interactions between parameters to be explored.38 Such analyses constitute a significant piece of work themselves and can make the object of subsequent manuscripts. In the current work, we focused on developing the model, calibrating it, and performing comparative analyses with respect to vaccination parameters under a few different vaccination scenarios; this already resulted in a large amount of information that needed to be interpreted and illustrated in a coherent and concise manner in the present manuscript.

Further work here could also involve various additional analyses on model outcomes including investigating impact of the actual transmission matrix or contact patterns, which in itself could make the object of a separate piece of work. For example, Van Effelterre et al in ref. 39 conducted a comparison looking at the impact of the transmission matrix in the context of varicella using more established approaches, in a stand-alone dedicated paper. The work that we presented here is not intended as exhaustive or definitive, but rather as a proof of concept and a starting point.

In summary, we aimed to develop a more realistic CMV transmission modeling framework with novel features, bringing together in an integrated fashion qualitative and quantitative current knowledge related to the CMV infection. The work presented here is intended as a step toward more advanced frameworks that can more realistically reflect the biology and epidemiology of CMV infection. After calibration and validation against available CMV epidemiological data from the US, the proposed modeling framework was employed to assess the potential impact to reduce congenital CMV infection at population level via vaccination with a hypothetical CMV vaccine. In the analyses presented here, we tested some CMV vaccination scenarios that were considered a priori of potential primary interest in the context, in an attempt to illustrate some of the current challenges associated with developing and implementing a CMV vaccine.40

Our results are based on a number of assumptions which we believe adequately reflect the qualitative knowledge related to CMV infection at present, with model calibration performed as a complex constrained optimization problem where all available relevant information is translated into corresponding model constraints to enforce biological and epidemiological plausibility. However, further studies and analyses are needed for more robust testing and validations in order to increase the level of confidence necessary for such modeling and simulation tools to further help inform decision making. In the absence of stronger evidence that can help select the most appropriate model, different modeling exercises can be conducted with potentially different outcomes.

Ultimately, projections from well validated epidemiological models will need to be evaluated from a cost-effectiveness point...
of view to complete the picture and support informed decisions. This was beyond the scope of the current work.

Finally, we want to acknowledge that the mechanistic deterministic modeling approach proposed here, while appealing because of its built-in tractability, has some limitations in the sense that everything is assumed as an average at the population level, with no explicit account for various sources of heterogeneity, variability or uncertainty. Alternatives such as individual-based models could be considered if finer levels of granularity (e.g., enhanced transmission in day-care settings, household transmission, differential impact of socio-economic status, individual risks, etc.) are of interest.

**Materials and Methods**

**Overview of the mathematical model**

The dynamic transmission modeling framework presented here is mechanistic and deterministic. The model structure, with underlying states and corresponding flows as illustrated in Figure 7, is designed to reflect qualitatively the known biology of CMV infection. The US population is stratified by gender and by vaccination status (vaccinated/not vaccinated). For each of these sub-populations, the model is further stratified to account for the natural history of CMV, using the following mutually disjoint states: susceptible (seronegative), primary infected (infectious, seropositive), latent (seropositive, non-infectious), endogenously reactivated (seropositive, infectious), exogenously re-infected (seropositive, infectious). For completeness, we also include a state allowing for the possibility (albeit small) that some seropositive individuals may be both reactivated (endogenously) and re-infected (exogenously).

**Modeling assumptions**

**Baseline model**

- The average duration of infectiousness $1/\alpha(a)$ was modeled as a parametric function of age, with an 18 months average duration up to 5 y of age, followed by an exponential decline so that, by the age of 12 years, duration of infectiousness plateaus at 4–6 months. The actual parameter governing the rate of exponential decline is estimated at calibration. For simplicity here, in a first approach and in the absence of compelling evidence, we assumed equal durations of infectiousness for primary and recurrent infections in the average population at calibration. This assumption is supported in part by findings in ref. 22 that suggested similar shedding patterns in CMV seropositive women with primary and recurrent infections. The theoretical modeling framework, however, readily enables a reduction factor to account for potentially shorter durations of infectiousness in recurrent vs. primary infections, which can be considered in the future as supported by evidence.

| $\alpha(a)$: the age variable | $\tau$: the time variable | Unvaccinated | Vaccinated |
|------------------------------|---------------------------|--------------|------------|
| Force of infection for primary infection | 1. $\lambda(a,\tau)$ | $1^*.$ $(1-V_C)\lambda(a,\tau), 0 \leq V_C \leq 1$ |
| Rate of progression to latency after primary infection | 2. $\alpha(a)$ | $2^*.$ $\alpha(a)$ |
| Endogenous reactivation rate | 3. $\gamma(a)$ | $3^*.$ $\gamma(a)$ |
| Force of secondary infection | 4. $(1-k)\lambda(a,\tau), 0 \leq k \leq 1$ | $4^*.$ $(1-V_C)(1-k)\lambda(a,\tau)$ |
| Rate of return to latency after reactivation | 5. $\alpha(a)$ | $5^*.$ $\alpha(a)$ |
| Rate of return to latency after re-infection | 6. $\alpha(a)$ | $6^*.$ $\alpha(a)$ |
| Force of secondary infection in reactivated | 7. $(1-k)\lambda(a,\tau), 0 \leq k \leq 1$ | $7^*.$ $(1-V_C)(1-k)\lambda(a,\tau)$ |
| Endogenous reactivation rate in re-infected | 8. $\gamma(a)$ | $8^*.$ $\gamma(a)$ |
| Rate of new infection inactivated in reactivated re-infected | 9. $\alpha(a)$ | $9^*.$ $\alpha(a)$ |
| Rate of reactivation inactivated in reactivated re-infected | 10. $\alpha(a)$ | $10^*.$ $\alpha(a)$ |

**Figure 7.** Model structure with compartments, states and flows. Details are given in the accompanying table below the figure.
– To account for the fact that CMV reactivation may increase with age, the rate of endogenous reactivation $\gamma(a)$ is allowed to be age-dependent as well. Here we model $\gamma(a)$ as a parameterized Gompertz-type function, which qualitatively enables reactivation to be flat and low in younger age groups, while allowed to eventually increase later in life. Three parameters involved in the mathematical expression of this function are estimated at calibration.

From a qualitative biological point of view, the choice of such an S-shape for the reactivation was based on the hypothesis that the potency of the immune system to control viral replication in the event of a reactivation changes with age, with relatively higher ability of the immune system to control the virus early in life, and which gradually becomes less and less potent leading to more frequent reactivation events or even a state of chronic infection in older individuals. While we acknowledge the absence of detailed quantitative data demonstrating this, we found numerous qualitative elements from the immunosenescence research field that are supportive of such an assumption (e.g., refs. 29, 30). Over lifetime, the CMV-specific T cells appear to become gradually less and less efficient in containing the viral replication (accumulation of high avidity clones with exhausted/dysfunctional profile and progressive accumulation of lower avidity clones that in turn also become dysfunctional). From this understanding of the immunological control of the viral replication, we concluded it is likely that earlier in life, the immune system is able to control reactivations more efficiently than later on (e.g., $> 60$ y of age). Thus, an S-shaped function, characterized by a gradual increase from low levels at young ages to higher levels at old age, appears to be more biologically plausible for reactivation than just a constant rate across all age groups. A flat (constant) reactivation is just a particular (simpler) case that has been considered in other models.

– CMV is transmitted through direct close physical contact of a susceptible person with oral fluids from infected individuals actively shedding the virus. CMV transmission is facilitated by social mixing. In order to capture adequate mixing between people of different ages, we employed an empirical social contact matrix (based on POLYMOD, courtesy of Dr. Niel Hens, Hasselt University, Belgian data) for close physical contacts longer than 15 minutes. We assumed that sexual interactions are implicitly represented in this type of contacts. Such empirical matrices are employed here only as a mean to render contact patterns, assuming similar social contact patterns in Western European countries and the US and scaling appropriately for the US age-stratified demography. While the POLYMOD-type empirical social contact matrices can provide a basic idea about the frequency of social contacts between individuals in different age groups, they do not reflect more infection-specific age characteristics. To take this aspect into account, the frequency of social contacts between individuals in different age groups can be multiplied by a “proportionality factor,” generally modeled as a bivariate parametric function $q = q(a, \tilde{a})$ ($a$: age of susceptible individual, $\tilde{a}$: age of infectious individual) related to infection-specific (susceptibility/infectivity) and setting-related characteristics. In the present work, we modeled this factor as a linear increase with respect to the age of the susceptible multiplied with an exponential decay with respect to the age of the infectious: $q(a, \tilde{a}) = (q_1 + q_2 a) e^{-a \tilde{a}}$, characterized by 3 parameters that are estimated at calibration. This assumption was guided in part by the age-stratified US seroprevalence data profile, where an almost linear increase for the CMV seroprevalence as function of age in the US population is apparent, and by the implicit assumption that likelihood of the infectious individuals to spread the infection decreases with increasing age (e.g., better hygiene habits). As this proportionality factor is estimated at calibration by fitting model outcomes against existing data, it ultimately plays the role of a corrective factor for the empirical social contact (mixing) matrix pattern in practice, resulting in a final transmission matrix that will also implicitly reflect setting- and infection-related characteristics.

– As considerable evidence indicates that seropositivity induced by a wild-type virus may provide protection against re-infection, we modeled this through a corresponding natural reduction factor $k$ in the force of infection for re-infection, as illustrated in Figure 5. This reduction factor is estimated at calibration.

– Congenital CMV infection is modeled through a risk function with 2 parameters accounting for the mother-to-fetus transmission rate following primary maternal infection vs. recurrent maternal infection, respectively, estimated at calibration. Age-specific fertility rates, included as customary in the underlying demographic model based on US Census data, are used to account for the impact of women’s age on the corresponding birth rate.

In summary, for the baseline model, our current framework involves 10 model parameters, whose point-values are estimated at calibration. This is discussed further below.

**Vaccination model**

One of the goals of this work was to employ a complex modeling framework, designed to reflect the biology and epidemiology of CMV infection, toward exploring the potential impact of a vaccine aimed primarily at preventing CMV infection to subsequently reduce the burden of congenital CMV infection at population level.

The ‘Vaccinated’ component in the model structure replicates the states and corresponding flows from the baseline model, as illustrated in Figure 7.

– In a base-case framework, the vaccine mode-of-action would be to reduce the risk of a susceptible vaccinated individual to acquire infection compared to a susceptible unvaccinated individual. Then the ‘vaccine efficacy’ ($V_e$) denotes a corresponding reduction in the risk of acquiring infection if vaccinated.

– The modeling framework is sufficiently versatile to enable consideration of various other potential vaccine modes-of-action, like reduced reactivation rates or reduced infectiousness in
vaccinated compared to unvaccinated individuals, or lower mother-to-fetus transmission in vaccinated infected mothers compared to unvaccinated infected mothers. Our base-case vaccination framework illustrated in Figure 7 corresponds to a situation where the vaccine has no impact on reactivation and where all the corresponding flows in the 'Unvaccinated' and 'Vaccinated' are similar except for a reduction in the corresponding force of infection in the 'Vaccinated' component, as highlighted in the explanatory table in Figure 7. For completeness, however, in our actual simulations we also considered an optimistic vaccination framework where the vaccine would have similar efficacy against reactivation as well, modeled as a corresponding reduction in the reactivation rate in the vaccinated seropositive individuals.

Mathematical framework
We employed a mathematical model formulation with continuous age structure and governed by a corresponding set of nonlinear Partial Differential Equations with respect to time and age, as illustrated in Supplemental File 2.

All the outcomes presented are based on corresponding numerical solutions with an age discretization of 1 year-step for all ages > 1 y old and < 86 y old (which is the assumed life expectancy in this case), and a 6 months-step for the 0–1 y old segment.

Model calibration and validation
Nationally representative age-stratified CMV seroprevalence data for the US have been collected as part of the National Health and Nutrition Examination Survey (NHANES) system and are currently published for two time periods: 1988–1994 and 1999–2004.25 As no major differences in CMV seroprevalence were observed between these 2 time periods, for simplicity, we assumed endemic equilibrium and we calibrated the baseline incidence were observed between these 2 time periods, for simplicity, we assumed endemic equilibrium and we calibrated the baseline parameter estimation problem here is formulated as a complex constrained optimization problem. Available qualitative and quantitative information on the age-stratified CMV seroprevalence curve in infants in the first year of life and overall consistent with the 1988–1994 NHANES III CMV seroprevalence data for the individuals aged 6–10 y, can be used as a check-point to provide additional information on the age-stratified CMV seroprevalence curve in infants (0–1 y of age).

As described in the Modeling assumptions, 10 model parameters have to be estimated at calibration. The parameter estimation problem here is formulated as a complex constrained optimization problem. Available qualitative and quantitative information is translated into appropriate mathematical constraints used to ensure both biological and epidemiological plausibility and to reduce the likelihood of over-fitting.

The constraints used for model calibration are based on the most recent publicly available estimates related to CMV infection in the US.9,11 Model projections here are considered plausible when leading to 1–4% of seronegative pregnant women experiencing a primary infection2 with a subsequent mother-to-fetus transmission rate of 30–40%,9 while simultaneously estimating a percentage of seropositive mothers with CMV-positive newborns around 1%11 with an overall target birth prevalence of congenital CMV infections set at 0.67%.9 Regarding the natural reduction k in the risk of an individual to acquire a new infection, due to natural immunity, we varied it between 60% and 70% at calibration, guided by information in refs. 3, 51.

In addition, we also considered the value of the force of infection among the US population ages 12–49 y as an additional soft constraint, estimated at a point value of 1.6/100 (with upper bound for the 95% confidence interval around 2/100) per year in ref. 2 which was also based on the 1988–1994 NHANES III CMV seroprevalence data in conjunction with a statistical model.

All our numerical simulations are performed in MATLAB 2010b, the MathWorks, Inc., Natick, Massachusetts, United States.

Disclosure of Potential Conflicts of Interest
All authors are employed by the GSK group of companies and own stock options in GSK.

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Authors’ Contributions
All authors contributed to define the objectives and global model structure. C. Hogea designed the model structure and the underlying mathematical and computational tools. C. Hogea and I. Dieussaert participated to the collection and assembling of the data. C. Hogea, I. Dieussaert and J. Mols performed and/or supervised the analysis. All authors had full access to the data, contributed to the interpretation of the results, reviewed and approved the present manuscript for submission.
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