Lethal *Streptococcus pyogenes* post-partum sepsis: Molecular analysis of an outbreak

Claire E Turner¹, Matthew Dryden², Matthew T G Holden³, Frances J Davies¹, Richard A Lawrenson¹, Leili Farzaneh¹, Stephen D Bentley³, Androulla Efstratiou⁴, Shiranee Sriskandan¹#.

¹Infectious Diseases & Immunity, Imperial College London, Hammersmith Hospital, Du Cane Road, London, W12 0NN, UK, ²Royal Hampshire County Hospital, Winchester, Hampshire, SO22 5DG UK, ³Pathogen Genomics, The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA UK, ⁴Microbiology Reference Services Division, Public Health England (formerly Health Protection Agency), Colindale, London, NW8 5EQ, UK.

#Author for correspondence:

Prof. Shiranee Sriskandan FRCP PhD

The National Centre for Infection Prevention and Management

Department of Infectious Diseases & Immunity

Imperial College Faculty of Medicine

Hammersmith Hospital, Du Cane Rd, London W12 0NN

Tel +44 (0)208 383 3135/3243; Fax +44 (0)208 383 3394

s.sriskandan@imperial.ac.uk

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Abstract

Sepsis is now the leading direct cause of maternal death in the UK and *Streptococcus pyogenes* the leading pathogen. We combined conventional and genomic analysis to define the duration and scale of a lethal outbreak. Two *S. pyogenes* post-partum deaths occurred within 24h; one characterized by bacteremia and shock, the other by hemorrhagic pneumonia. The women gave birth within minutes of each other in the same maternity unit 2d earlier. Seven additional infections in healthcare and household contacts were subsequently detected and treated. All cluster-associated *S. pyogenes* isolates were genotype *emm*1 and indistinguishable from other UK *emm*1 isolates. Sequencing of the virulence gene *sic* revealed that all outbreak isolates had the same unique *sic*-type. Genome sequencing confirmed the cluster was caused by a unique *S. pyogenes* clone. Transmission between patients occurred on a single day, and associated with casual contact only. A single isolate from one patient demonstrated a sequence change in *sic* consistent with longer infection duration. Transmission to healthcare workers was traced to single clinical contacts with index cases. The last case was detected 18d after the first case. Following enhanced surveillance the outbreak isolate was not detected again. Mutations in bacterial regulatory genes played no detectable role in this outbreak, illustrating the intrinsic ability of *emm*1 *S. pyogenes* to spread while retaining virulence. This fast-moving outbreak highlights *S. pyogenes* potential to cause a range of disease in the puerperium with rapid transmission, underlining the importance of immediate recognition and response by clinical infection and occupational health teams.
Introduction

Cases of post-partum maternal *Streptococcus pyogenes* sepsis occur sporadically; paired cases and deaths are rare in the developed world (1, 2). We describe two post-partum deaths due to *S. pyogenes* (group A *Streptococcus*, GAS) that occurred within 24h in the same maternity unit; one case was characterised by septic shock and the other by haemorrhagic pneumonia. Both cases were associated with seven additional infections detected in healthcare and family contacts. We combined genotypic and phenotypic analysis with whole-genome sequencing to confirm the isolates were unique and distinct from other *emm*1 GAS isolates circulating in the UK, enabling us to determine the time limits and scale of this outbreak.

Case reports

Case 1

A 39y old teacher (G3P2) presented at term with irregular contractions and was monitored for 5h in the maternity unit prior to discharge. She was readmitted 5h later and delivered a live female infant. Other than a small second-degree tear, there were no complications and she was discharged 13h after delivery. Approximately 30h following delivery she awoke with lower abdominal pain necessitating hospital re-admission, inhaled nitrous oxide and opiates, although physiological measurements were initially normal. She collapsed, 6h later, with hypotension and was transferred to the intensive care unit. She rapidly developed multiorgan failure, and responded poorly to inotropic and ventilatory support in addition to broad spectrum antibiotics. The following day, blood cultures indicated a streptococcal infection and high-dose intravenous pooled human immunoglobulin G (IVIG) was administered. The patient deteriorated and died, 2.5d after delivery of her third child. GAS was isolated from
blood cultures and genital tract swabs obtained ante-mortem and also from uterine cervix tissue obtained post-mortem.

Case 2  
A 29y old primigravid teacher was admitted in labour to the same unit, on the same day as case 1. She had experienced spontaneous rupture of membranes 48h earlier, and had commenced prophylactic amoxicillin. During the day she had had a transient temperature of 37.7°C which settled, though other physiological measurements were normal. An oxytocin infusion was commenced and 12h later, a live male infant was delivered by emergency caesarean section and a final antibiotic dose was administered intra-operatively. The time of caesarean delivery was two minutes before the delivery of case 1’s baby, though in a separate section of the maternity unit and undertaken by different staff. She was discharged approximately 66h after caesarean section. Approximately 76h after delivery, she developed a cough and chest pain associated with blood-stained sputum. A community midwife arranged hospital re-admission. While packing, the patient collapsed in respiratory arrest associated with haemoptysis. Resuscitation was attempted by a paramedical team on site, and in the hospital emergency unit but the patient died on Christmas Eve, 3.5d after caesarean section. GAS was isolated from throat, haemorrhagic lung and uterine cervix samples post-mortem, but not from the caesarean wound or lower genital tract. GAS isolates from both cases were subsequently identified as emm-type 1.

Response of infection team and intensive surveillance  
A hospital outbreak control team managed the incident, surveillance and epidemiology. Household and healthcare worker (HCW) contacts of each patient were screened, including antenatal, during labour and postnatal contacts (Figure 1). Environmental screening was
conducted once in the immediate aftermath of the outbreak, by swabbing baths, showers, basins, bed space surfaces and these were cultured on blood agar.

As part of a two month period of intensive surveillance, all mothers who had been on the unit concurrently with the two cases were contacted and advised to seek medical attention and screening if symptomatic. All women in labour were screened during this period within a day of admission. Throat and skin swabs (if skin lesions reported) were used for screening for GAS, plus lower genital tract swabs for all women in labour. During the intensive surveillance period, enhanced twice daily chlorine-based cleaning in the maternity unit was undertaken (previously once daily detergent-based cleaning).

**Screening results**

**Household contacts**

Four household contacts of case 1 were screened, of which 2 were positive for GAS. Two household contacts of case 2 were screened, of which 1 was positive for GAS.

The baby of case 1 was admitted to the neonatal unit with a raised CRP and received ceftriaxone for suspected sepsis; nose and ear swabs obtained 2d after delivery yielded GAS *emm*1, though blood and CSF cultures were negative. The baby of case 2 was not colonized or infected with GAS.

Throat swabs from the partners of case 1 and case 2 yielded GAS *emm*1 and both received antibiotics. One partner reported pharyngitis at the time of screening (i.e. after the death of case 1) while the other developed a lower respiratory tract infection necessitating hospital admission 4d after the death of case 2.

**Healthcare contacts**
Of 69 HCW contacts screened, 3/69 (4.3%) had throat swabs that yielded GAS emm1, and each received antibiotic treatment. The three HCWs reported single episodes of postnatal clinical contact with the patients, and included an obstetrician who examined case 1, an ICU nurse present at the intubation of case 1, and a community midwife attending case 2 at home. Two of the HCWs developed significant symptoms 7-10d after the deaths; one had tonsillitis and cervical lymphadenopathy while the other had pharyngitis. HCW who tested positive for GAS were offered 10 days treatment with oral amoxicillin, removed from work for this period and were screened again at the end of treatment. The partner of a symptomatic HCW was treated for GAS pharyngitis, though the isolate was not available for study.

193 HCWs from the same institution, who were not contacts of the two cases, were also screened. 2/193 (1%) had throat swabs that yielded GAS and received antibiotics; however these isolates were emm12.

Other patients
In the immediate aftermath of the outbreak, 42 maternity patients were screened, including women who reported symptoms compatible with GAS and had been on the unit at the same time as the two fatal cases, along with those admitted up to two weeks later. 1/42 had a throat swab that yielded GAS, typed as emm1. This patient reported pharyngitis and sinusitis that developed one week post-partum and also recalled meeting and talking with case 1 on the day of her original admission (Figure 1). She was treated with antibiotics in the community.

Two further cases of invasive GAS infection (necrotizing fasciitis and pneumonia) were admitted from the community during the month following the maternal fatalities; however these GAS isolates were emm75 and emm89 (Figure 1).

Environment
GAS was not isolated from any environmental samples.
Enhanced surveillance

A further four month period of enhanced clinical surveillance was instituted within the obstetric and neonatal units in which HCW were particularly alert for all GAS infection and swabbed throats and skin of patients or HCW if there was any history or indication of inflammation. No further GAS carriage or infection was detected.

Materials and Methods

GAS strains: phenotyping and genotyping

All 15 emm1 GAS isolates from the cluster were compared with 14 other emm1 GAS isolates submitted to the national reference laboratory from elsewhere in England in the three months preceding and one month following the outbreak, including, where available, isolates from the same region (Table S1). GAS phenotypic comparison, emm-typing, superantigen genotyping and sequencing of covR/S and sic loci are described in the supplementary methods.

GAS whole-genome sequencing

Illumina sequencing was performed on 24 strains, including all 15 strains from the outbreak, and mapped against the complete genome sequence of the USA emm1 strain MGAS5005 (3). In excess of 50-fold sequence coverage was achieved for all isolates (Supplementary methods).

Measurement of anti-emm1 GAS immunity in pregnant women

As a surrogate for immunity among women of child-bearing age in the UK, anonymized antenatal screening serum samples were obtained from Imperial College Healthcare Trust and used in accordance with approval from the West London Research Ethics Committee.

ELISA-based assays of immunity
Reactivity of antenatal sera towards recombinant SIC proteins was tested using an ELISA-based method. Diluted antenatal sera were applied to SIC protein-coated wells and bound IgG was detected with goat antihuman IgG-HRP. In order to quantify SIC-reactive IgG in each serum sample relative to the human blood product intravenous immunoglobulin (IVIG, Zenalb 4.5) that has high anti-streptococcal activity, the activity of IVIG against SIC protein was also measured using a series of fixed concentrations of IVIG to generate a standard curve of IVIG-equivalent antiSIC IgG on each plate. A similar protocol was used to detect IgG reactivity of antenatal sera towards whole emm1 S. pyogenes cells but the wells were coated with heat-killed emm1 S. pyogenes. Details are provided in the supplementary methods.

Opsonophagocytosis assays of immunity

Assays were performed using 80 (of 199) representative heat-inactivated antenatal patient sera. Eemm1 GAS (strain H584) were stained with fluorescein isothiocyanate (FITC, Invitrogen) and opsonised with test serum before adding to 2x10^6 fresh human neutrophils and 10% complement (rabbit serum, Merck Chemicals, UK). Neutrophils with internalized FITC-labelled bacteria were measured by flow cytometry. To exclude external adherent FITC-labelled S. pyogenes samples were quenched with trypan-blue. Antenatal-serum-opsonised GAS were compared with IVIG (2.5mg/ml)-opsonised GAS, as this has been shown to provide optimum opsonophagocytosis. Non-opsonised GAS were used as a negative control.

Results

Phenotypic analysis of GAS isolates

To determine whether the 15 emm1 GAS isolates from the maternity unit exhibited excessive virulence, they were compared phenotypically with 14 other emm1 strains from the UK using a wide panel of in vitro analyses that included growth in whole blood, expression of capsule,
the chemokine protease SpyCEP, cysteine protease SPEB, and superantigenicity (Figure S1). There were no phenotypic differences detected between the outbreak cluster and other emm1 strains circulating in the UK. Isolates were penicillin sensitive and the MIC did not differ from other emm1 isolates (0.03mg/L).

**Molecular analysis of GAS isolates**

Although 5-10 different superantigen genotypes have been described in European emm1 strains (4), all 29 outbreak and non-outbreak strains had the same superantigen genotype (speA, speG, speJ, smeZ).

Sequencing of the streptococcal inhibitor of complement (SIC, encoded by sic), identified 13 different sic alleles among all 29 emm1 isolates studied, the most common allele identified being sic1.02 (Table S1). The 15 maternity unit emm1 isolates all, with one exception, had the same but unique sic gene, designated sic1.300. One genital tract isolate from case 1 demonstrated a further unique sic allele, designated sic1.301. As sic1.301 differed from sic1.300 by an 87bp deletion (Figure S2), we surmised that sic1.301 had arisen from sic1.300 during mucosal carriage indicating possible longer duration of infection (5, 6).

Despite distinctive sequences, there was no difference in the ability of recombinant variant SIC proteins (SIC1.300, SIC1.301, and the common UK-type SIC1.02) to inhibit complement-mediated erythrocyte lysis (Figure S2), consistent with published structure-function data (5, 7).

**Whole-genome sequencing**

Whole-genome sequencing of the 15 maternity unit emm1 isolates and nine of the 14 unrelated emm1 isolates from elsewhere in England was undertaken to determine if any features distinguished the isolates in addition to the sic genotyping.
Genome sequencing confirmed that the 15 maternity unit strains were unique and differed from the nearest GAS relative by eight core genome single nucleotide polymorphisms (SNPs); six of these SNPs were unique to the outbreak cluster (Figure 2). Of the six unique SNPs, one was non-synonymous and occurred in the *sic* gene (consistent with the previously noted unique *sic* gene sequence). Additionally, outside of the core genome, the outbreak cluster had a unique non-synonymous SNP that mapped to a gene of unknown function in phage 5005.2.

Comparing all 24 sequenced *emm*1 strains with the MGAS5005 genome, the most recently sequenced USA *emm*1 strain (3), a total of 250 SNP loci were identified within the core genome (excluding phage-related sequences), of which 138 (~55%) were nonsynonymous substitutions, 80 (~32%) synonymous substitutions, and 32 (~13%) were within intergenic regions (Tables S2, S3 and S4).

Mutations in GAS regulatory genes, reported to be associated with strain ‘hyper-invasiveness’ were not seen in any of the 15 maternity unit isolates. Unique mutations in *covR/S* and *rgg* were seen only in 2/7 and 1/7 invasive *emm*1 strains from elsewhere in England respectively and these were associated with predicted phenotypic changes that included increased SpyCEP and capsule, but reduced SPEB expression (Figure S1).

During the short outbreak course, no new SNPs arose in the 15 maternity unit isolates. However, in addition to the *sic* deletion mutation already detected in a genital tract isolate from case 1, a truncation deletion mutation in the capsule biosynthesis gene, *hasB*, was identified in a pharyngeal isolate cultured from the maternity patient contact with 11d history of pharyngitis. This had no measurable impact on capsule produced *in vitro* (Figure S1).

**Immunity to *emm*1 GAS in antenatal population**
To investigate antenatal population immunity to *emm*1 GAS, 199 sera were tested for reactivity to each SIC allele (*sic* being specific to *emm*1) and to *emm*1 GAS surface proteins. Of the 199 sera, 7% showed no recognition of SIC, while a majority showed low reactivity and a small proportion (8%) showed strong reactivity similar to IVIG. SIC allele-specific responses were seen only in a minority (Figure S3). Towards GAS surface proteins, only 16% of the 199 antenatal sera had any detectable antibody (Figure S4).

The ability of antenatal sera to opsonize FITC-labelled *emm*1 GAS was determined by co-incubation with fresh human neutrophils and measurement of subsequent phagocytosis; 5% of samples demonstrated no ability to facilitate opsonophagocytosis of *emm*1 GAS, while only 4% showed activity equivalent to that seen when testing human IVIG (Figure 3).

**Discussion**

This lethal outbreak of *emm*1 *S. pyogenes* in a maternity unit was explosive, commencing with a likely community source. Transmission events occurred on a single day with apparently transient contacts. Whole genome sequencing confirmed cases to be clonal, although events were so rapid that a chronological sequence of transmission could only be determined by changes in a virulence gene, *sic*, rather than conventional analysis of whole genome data.

The rapid and lethal nature of this outbreak highlights a number of learning points. Firstly, GAS infection remains a deadly threat in the puerperium. The transmission risk within the maternity setting is high, not only to HCWs who attend infected patients, but also between social and family contacts, and to the newborn infant. Oropharyngeal carriage may be important and single episodes of contact are sufficient for productive transmission to occur (including both social and healthcare contact). Recognized risk factors for post-partum sepsis may not always be present, and signs of severe sepsis may be masked or present atypically.
Invasive GAS infection in the post-partum period is not limited to classical genital tract (puerperal) sepsis and may present to physicians other than obstetricians, progressing rapidly to irreversible multiorgan failure.

Remarkably, sepsis is now the UK’s leading cause of ‘direct’ maternal deaths. GAS accounted for 13 of 25 sepsis-related maternal deaths reported in 2006-8 by the UK Centre for Maternal and Child Enquiries (CMACE) although the reasons for the increases are unclear (8, 9). Although pairs of GAS infection caused by the same emm-type have been previously reported in maternity units, such events are rare and may be related to HCW carriage (1, 10, 11). The outbreak described was caused by emm1 GAS, the major lineage associated with invasive disease in Europe and the USA. Although GAS puerperal sepsis is most frequently associated with emm28, the mortality of GAS puerperal infection is disproportionately attributable to emm1 and emm3 S. pyogenes isolates (12). Environmental factors that influence GAS outbreaks have been well documented (seasonal variation, sharing rooms, hygiene etc) (13-16) but the host and the pathogen factors affecting transmission of S. pyogenes in this setting are unknown.

The affected women were admitted in labour, to the same unit, on the same day. It is not possible to definitively determine whether infection was acquired outside the hospital and then transmitted sequentially person-to-person within the unit, or whether the maternity patients acquired their infection from a common source. However, one isolate (out of four) from case 1 had undergone allelic change in sic at an unknown time point, indicative of longer mucosal colonization. Changes in sic within individual patients carrying GAS mucosally have been reported previously, as have changes during epidemics within a population (5, 17), but this may require prolonged carriage (>2 weeks); indeed experimental mucosal infection of mice failed to induce allelic change in sic over 9d (not shown).
consistent with other studies (5, 6). Due to the high level of repeat regions within the \textit{sic} gene, the mapping software used to analyse whole genome sequencing cannot identify such changes, necessitating conventional sequencing. Although both women delivered within minutes of one another, symptomatic infection in case 2 arose 2d later than case 1; we speculate that administration of prophylactic beta lactam antibiotics for ruptured membranes may have delayed onset of disease, although were insufficient to prevent GAS acquisition or colonization.

The routes of GAS transmission in the puerperium are hard to investigate, since samples are seldom obtained from women antenatally, or from contacts, except where an outbreak occurs. GAS is rarely present in the genital tract antenatally (18-21), hence screening or clearance regimes, antenatally or intra-partum, are unlikely to impact on post-partum GAS infection. The importance of the respiratory tract as a source of infection to recently pregnant women was established in the 1930’s, and re-iterated in the CMACE report (8, 18). GAS throat carriage among healthy adults in England is low, around 0.5-2\% (22) consistent with the 1\% carriage rate (of non-outbreak strains) we detected in HCWs who were not direct contacts of the cases. In contrast, 4.3\% of the HCWs identified as contacts were found to be carriers or infected with the outbreak strain suggesting that it may be especially suited to spreading in this setting. Transmission of the outbreak strain to HCWs was however limited to those reporting close but single clinical contacts and raises the question as to whether mask wearing is advisable for HCWs treating GAS patients. Screening of HCWs was performed using throat or skin swabs thus excluded those carrying GAS rectally or vaginally. Previous outbreaks have been associated with HCWs asymptatically carrying GAS at all mucosal sites (11) therefore we cannot fully exclude an unknown HCW source of GAS. HCW compliance is essential during outbreak investigation (13, 23) and newly issued UK guidance
(14) recommends nasal, rectal and vaginal swabs should be obtained if other swabs are negative, in situations where a HCW is implicated in transmission.

Contact with children either in the home or workplace has been reported in association with maternal invasive GAS infection (8); coincidentally, both women who died were teachers, although they taught at separate schools. While it is possible that children represented a reservoir for the outbreak strain, the strain was not detected in any of 193 HCW that were not contacts of the patients, nor was it identified among 100 GAS throat isolates submitted from the affected region in the one year following the outbreak. We therefore conclude that the outbreak strain was not circulating in the wider community.

Women are 20 times more susceptible to hemolytic streptococcal bacteremia after delivery compared to non-pregnant women; this risk increases to 100-fold if all invasive GAS infections are included (24, 25). There is a pressing need to establish a robust assay for GAS immunity that can be applied to large populations. We used an assay that solely measured serum opsonic function that could result in phagocytosis, to determine immunity in a similar but unrelated antenatal population. 5% of antenatal sera showed no opsonic activity against emm1 GAS, highlighting an underlying susceptibility to GAS infection in a significant proportion. Whether immunity to GAS alters during the course of pregnancy is entirely unknown and a subject of ongoing research.

To our knowledge, the emm1 GAS isolates in this study are the first outside of the USA to be sequenced. We chose to align our sequences against the emm1 genome strain MGAS5005 for comparison and SNP identification (rather than SF370) as it is more representative of the globally disseminated emm1 clone (3). Mutations in regulatory genes are reportedly characteristic of emm1 hyper-invasiveness (26-28), but may result in fitness cost, reducing colonization potential (29). These mutations were not observed in any of the outbreak strains,
and in less than half of invasive emm1 strains from elsewhere in England. Preservation of
GAS regulatory gene function may have underpinned the rapid transmissibility and
adaptability essential for this explosive but short-lived outbreak.

This outbreak illustrates the devastating rapidity and intensity with which GAS can spread
within a susceptible population and draws attention to the need for urgent infection control
intervention, including immediate staff screening in a suspected outbreak to prevent onward
transmission events. Staff screening should occur prior to results of molecular typing being
available and, ideally before staff return to duty, although this may necessitate a flexible
approach to the screening process rather than conventional use of occupational health units.

Emm1 GAS is equipped with a range of virulence factors that allow it to cause the spectrum
of disease observed in this outbreak (30), from asymptomatic colonization, through to
tonsillitis, hemorrhagic pneumonia, and bacteremia toxic shock. Recent guidelines regarding
outbreak prevention and management of severe sepsis in the obstetric patient are timely (31).
The cases emphasize the need for vigilance when reviewing maternity patients presenting
after childbirth with regard to suspicion of sepsis, which may develop in the community, as
occurred in both cases reported here.
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References


Figure Legends

Figure 1. Identification of Group A Streptococcus (GAS) cases. Cumulative number of individuals carrying or infected with the outbreak-strain by date (upper axis, blue shaded bars). Individuals with the outbreak-strain included maternity patients (solid shading), household contacts (diagonal cross-hatching), and healthcare workers (HCWs, horizontal cross-hatching). Individuals found to be carrying or infected with other GAS isolates during the period of surveillance are shown on the lower axis (grey bars). Period of overlapping hospital admission for three maternity patients denoted by grey shaded area. During the period of intense surveillance which started on December 24th and lasted for two months, two HCWs were identified to be carrying emm12 GAS (grey shaded, horizontal hatching bars) and two non-maternity patients had community-acquired invasive infections caused by emm89 (grey shaded bar, 3rd January) and emm75 (white bar, 23rd January) GAS strains. Enhanced surveillance continued for a further four months, during which time no further GAS was isolated from any maternity patient.

Figure 2. Phylogenetic analysis demonstrating that the core genomes of the maternity unit isolates were identical to each other but different to contemporaneous emm1 isolates submitted to the reference laboratory. The genomes of all 15 isolates from the maternity unit and nine emm1 isolates from around England were sequenced and mapped to the complete genome sequence of MGAS5005, a contemporary USA strain (3). Maximum likelihood phylogenetic tree generated from core genome single nucleotide polymorphisms (SNPs) of 24 emm1 isolates. The maternity unit isolates clustered in a single clade that differed from the nearest relative by eight SNPs. The sic allele for each isolate is indicated. The unique sic allele (sic1.301) that arose within the outbreak cluster is highlighted in red. The scale bar
represents substitutions per SNP site. Details of all SNPs are provided in the supplementary appendix (Supplementary table 2). HCW; healthcare worker, URT; upper respiratory tract, LRT; lower respiratory tract, GT; genital tract.

Figure 3. Immunity to emm1 GAS among healthy pregnant women. The ability of antenatal sera to opsonize FITC-labeled M1 GAS is shown for 80 representative sera (out of 199). Percentage of neutrophils associated with serum-opsonized FITC-labeled bacteria was measured by flow cytometry and expressed relative to the percentage of neutrophils associated with FITC-labeled bacteria when IVIG (2.5mg/ml) was used as the opsonin. Negative; below the level of the ‘no serum’ (complement alone) control (≤10% of IVIG-equivalent opsonophagocytosis).