Quantitative Longitudinal Antibody Monitoring in a COVID-19 Outpatient Case Series:

Relations of IgM/IgG Dynamics to Anosmia and Ageusia

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ABSTRACT

A case series of 22 patients with confirmed SARS-CoV-2 infection and Corona Virus Disease 2019 (COVID-19) symptoms was followed in a primary care clinic. We provided quantitative and longitudinal profiling for SARS-CoV-2 IgM and IgG with a point of care device. Half the patients had a history of anosmia or ageusia. IgM and IgG responses were highly heterogeneous quantitatively and temporally. We determined that clinical symptomatology of chemosensory loss correlated with lesser but sustained titers of IgM and IgG whereas normal chemosensation correlated with transiently higher but rapidly declining antibody levels.

KEY WORDS

Anosmia
Ageusia
Antibody monitoring
COVID-19

INTRODUCTION

Previous reports from China early in the Corona Virus Disease 2019 (COVID-19) pandemic on hospitalized patients indicated an early but variable antibody response. (Jin et al, 2020, Long et al, 2020, Zhao et al, 2020). In particular, IgM responses predated IgG, but there was substantial individual variability. There was no attempt to correlate the antibody profiling with early clinical symptoms in these early inpatient studies.

Anosmia is the absence of all olfactory function and can be caused by upper respiratory tract infections (Vroegop et al., 2020). Ageusia is the loss of taste of the tongue, particularly the inability to detect sweetness, sourness, bitterness, saltiness, and umami (Abduljabbar et al., 2020). Among the symptoms of COVID-19, the combination of anosmia and ageusia is pathognomonic for the disease (Walker et al., 2020). The virus may be causing damage to the olfactory pathway or may be targeting non-neuronal cells that express ACE2 receptors such as olfactory epithelium, microvillar cells and others (Sehanobish et al., 2021).

Studies from different countries have described anosmia as the most common symptom with a median time of presentation of 3-days post-infection (Gandica et al., 2020). Case reports showed a high rate of olfactory function recovery within 1-2 weeks after the onset of the symptom (Vaira et al., 2020a). The prevalence of these conditions in the COVID-19 pandemic is not a majority of patients, but rather, an upper bound of 20% (Vaira et al., 2020b).

To the extent that the symptom may represent an indicator of a higher infectious inoculum, we thought to assess patterns of immune response by measurement of IgM and IgG antibodies in COVID-19 patients who developed loss of taste and smell versus those who did not. We carried the study in a community clinic with a point-of-care device to prototype caregiver monitoring of seroprevalence for disseminated public health implementation. This report details the experience of a clinic in Coral Gables, Florida treating predominantly Hispanic patients from the onset of the COVID-19 pandemic.

METHODS

Patients. Patients were identified at the FastLabs (Coral Gables, Florida) from March to November 2020 and were asked to participate based on their willingness to donate a small sample of capillary blood and complete a short questionnaire. Patients signed an informed consent form agreeing to DNA testing and to the use of their de-identified information of a statistical nature for quality improvement, validation, research, and accreditation purposes.

A total of 22 COVID-19 positive patients were defined as those who had received a confirmed SARS-CoV-2 diagnosis by use of a RT-PCR on a respiratory tract sample. The date on their confirmed diagnosis was used as original infection date. Diagnoses must have occurred prior to the commencement of the study.

During the participants' initial appointment, all were asked to complete a questionnaire to capture basic demographics and COVID-19 related information: diagnosis details, reported symptoms, and recent viral tracing. The questionnaire was primarily used to evaluate the occurrence of 12 notable clinical manifestations of COVID-19 in patients, as follows: coughing, fever or chills, shortness of breath, loss of taste, loss of smell, fatigue, muscle or body aches, headache, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea. All patients provided consent prior to participation.

Quantitative Antibody Measurements. To obtain measures of SARS-CoV-2 antibodies, a quantitative point-of-care (POC) diagnostic device was required. FastLabs at Coral Gables, Florida has a CLIA Certificate of Waiver #10D2214812 from the Florida Agency for Health Care Administration and the Centers for Medicare and Medicaid. We utilized the Lansion LS-1100, a Dry Fluorescence Immunoassay Analyzer with its corresponding Time-Resolved Fluorescence Immunoassay (TRFIA) for quantitative antibody measurement (Lansion Biotechnology Co. Ltd., Nanjing, China). Time-resolved fluorescence is obtained by an excitation light source at 365 nm, with emitted light at 610 nm (Lansion Biotechnology, 2021).

For the antibody test, the TRFIA consists of the Lansion COVID-19 IgM & IgG Test Kit (Dry Fluorescent Immunoassay), a lateral flow cartridge read by the analyzer. The test measures human IgM and IgG antibody titers developed by an individual against the SARS CoV-2 virus spike protein (S) (Lansion Biotechnology, 2021). This protein is a type I transmembrane glycoprotein and mediates the entrance to human respiratory epithelial cells by interacting with cell surface receptor angiotensin-converting enzyme 2 (ACE2).

Capillary blood samples (finger prick) were obtained and placed in the TRFIA lateral flow assay, with Lansion's detection reagent at room temperature, allowing the binding of the antibodies to analyte fluorescent reporter molecules at a detectable wavelength. After a 15 minute reaction time, the assay is placed in the appropriate slot in the analyzer to gather a quantitative measurement of the antibodies on the assay, reported in international units per volume (mIU/ml).

Patient Follow up. After the initial measurement, COVID-19 positive patients were asked to return for a second quantitative assessment at a later date. The minimum time between antibody readings was 14 days in order to appropriately measure any changes in IgG and IgM antibodies.

There was a control cohort of 12 SARS-CoV-2 negative subjects without any COVID-19 symptoms who was tested as a reference for the assay.

RESULTS

Demographics. The case series cohort was predominantly Hispanic (20 Hispanics, 2 White) and male (13 men, 9 women). Median age was 37 years old. The control cohort was all Hispanic and predominantly female (8 women, 4 men). Median age was 45 years old.

Control measurements. The range of titers for the 12 individuals without a history of COVID-19 disease was from 0.01 to 0.07 mIU/ml (Mean 0.03) for IgM and from 0.00 to 0.09 mIU/ml (Mean 0.03) for IgG.

Symptoms. The most common symptom was fatigue, experienced by 14 participants (64%) followed by body or muscle aches, experienced by 11 participants (50%) *(**Figure 1**)*. Loss of smell was reported by 10 patients, loss of taste by 10 patients. There was coincidence of the chemosensory symptoms, as 9 patients reported both anosmia and ageusia. Loss of smell or taste was reported by 11. Headache or congestion was reported by 10 patients each (45%).

Antibody dynamics. The timing of antibody responses ranged from 31 to 198 days post-diagnosis. The period between initial and second measurements ranged from 13 to 55 days. There was wide variability among the patients with regard to IgM and IgG titers. However, there were distinctive patterns differentiating the patients with chemosensory deficits. Patients who lost either smell or taste had more sustained IgM and IgG titers than patients without. Difference in antibody titers and the time interval between readings for individual patients was examined to evidence visually the dynamics of immune response.

IgM antibody titers ranged from undetectable to 2.2 mIU/ml. The change in antibody titers between measurements ranged from -0.41 to +0.15 mIU/ml for IgM. Of the 11 patients in each group, IgM titers decreased in only 3 subjects in the chemosensory deficit group, compared to 8 in the group with normal chemosensation [X^2 (1, N = 22) = 4.5455, p = .03301]. *(**Figure 2**)*.

IgG antibody titers ranged from undetectable to 1.04 mIU/ml. The change in antibody titers between measurements ranged from -0.33 to +0.15 mIU/ml for IgG. Of the 11 patients in each group, IgG titers decreased in 5 subjects in the chemosensory deficit group, compared to 9 in the group with normal chemosensation [X^2 (1, N = 22) = 3.1429, p = .07626] *(**Figure 3**)*.

Total Antibody was calculated by adding IgM and IgG measurements in mIU/ml. Total antibody titers ranged from 0.16 to 1.99 mIU/ml. The change in antibody titers between measurements ranged from -0.71 to +0.21 mIU/ml for Total antibody. Of the 11 patients in each group, Total Antibody titers decreased in 4 subjects in the chemosensory deficit group, compared to 9 in the group with normal chemosensation [X^2 (1, N = 22) = 4.7009, p = .03015] *(**Figure 4**)*.

DISCUSSION

Dynamics and variability of IgM/IgG in COVID-19. Our observations evidence high variability in the dynamics IgM and IgG. There was no apparent separation in the appearance of the antibodies. Conventional expectations of increased in IgM titer preceding IgG were not observed. This finding suggests limitations in the assessment of the time of infection from relative amounts of IgM and IgG. It has been observed that a significant reduction of IgM is observed as SARS-CoV-2 nucleic acid is cleared in recovery. Yet serum IgG declined even more significantly, raising concerns on the sustainability of the humoral immune response (Zhou et al., 2021). There is however a direct correlation between the severity of disease and antibody response (Lynch et al., 2021).

We observe that the early clinical symptomatology is also relevant. In particular anosmia and ageusia correlate with more sustained titers of IgM and IgG. The course of natural infection with SARS-CoV-2 reflects a wide array of inocula in terms of viral amount and exposure time. The pathognomonic symptoms of chemosensory deficits (loss of smell or taste), a consequence of SARS-CoV-2 infection in the nasal and oral neuroepitheilium, may be markers for higher inocula and more sustained antibody response.

Limitations. POC serological tests, usually have a lower diagnostic performance compared with laboratory tests, particularly with a capillary blood sample in less controlled environments (Liu and Rusling, 2021). We believe that by extending the diagnostic testing window in the case series, measuring explicitly inter-individual variance, and longitudinal intra-individual fluctuations in antibody levels provides additional accuracy than a single isolated measurement.

The quantitative antibody profiling assay from a capillary blood specimen implemented in this report has not achieved designation as Emergency Use Authorization by the FDA. The POC device utilized in this study therefore remains a research assay. IgA antibodies, which are also prevalent in early SARS-CoV-2–specific humoral responses were not part of the assay utilized.

As a case series, this report by default is limited in sample size. The interval of longitudinal follow up was variable, and overall briefer in the patients with anosmia or ageusia, which could preclude definitive assessment of sustainability. The interval from COVID-19 diagnosis to serological testing was also variable, which confounds the analysis with disease stage. However, it is apparent that the patients with normal chemosensation developed higher and transient antibody titers compared to a lesser amount but sustained course in the patients with chemosensory deficits.

IgA antibodies, which are also prevalent in early SARS-CoV-2–specific humoral responses were not part of the assay utilized. Early and defined IgA responses have been reported in COVID-19 patients preceding even IgM (Dahlke et al. 2020). However, as IgA is a major agent in mucosal defense mechanisms against respiratory viruses, its detection in systemic capillary blood would be difficult.

Implications for public health. In general, antibody profiling historically has been a part of public health surveillance and seroprevalence as contrasted with clinical management for the individual. This work was performed in a primary care clinic with a point-of-care device.

Our results demonstrate it is feasible to perform antibody monitoring in this setting, which is valuable for public health. The world is facing a situation with substantial amount of pre-infected individuals receiving vaccines. Understanding the interplay of natural infection and vaccination

231   will be important for public health, estimating the level of community immunity, and surveillance
232   of new infections by variants of SARS-CoV-2.
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ACKNOWLEDGMENTS

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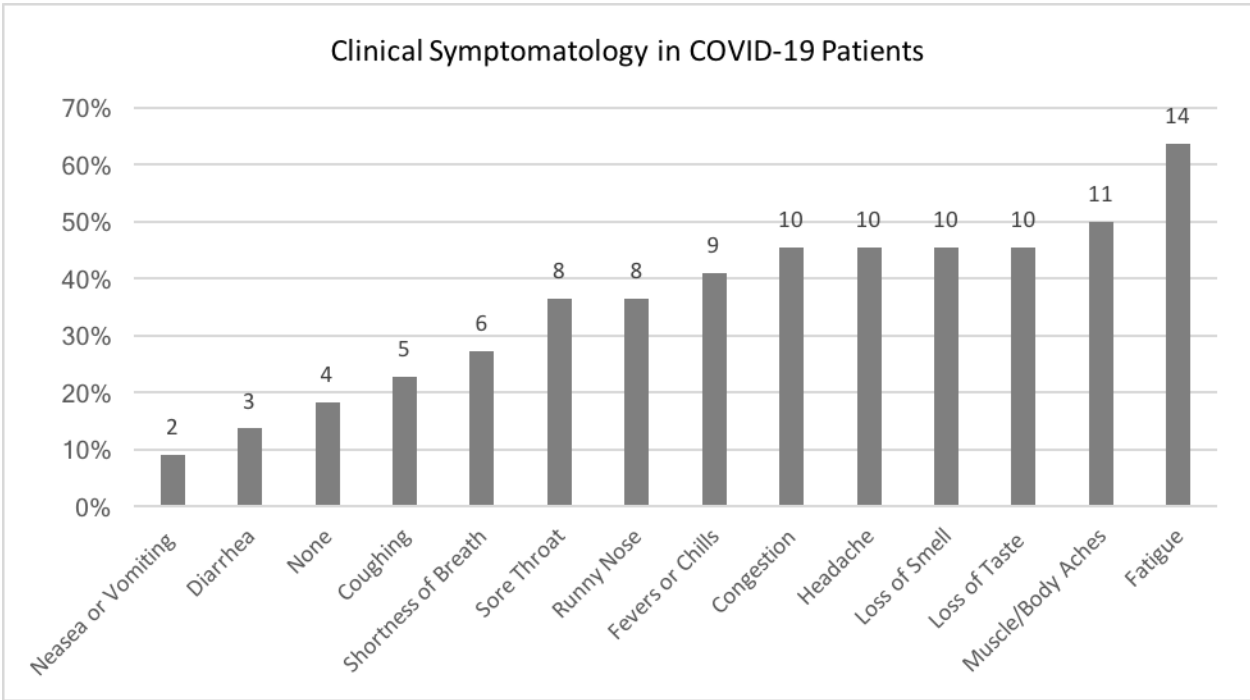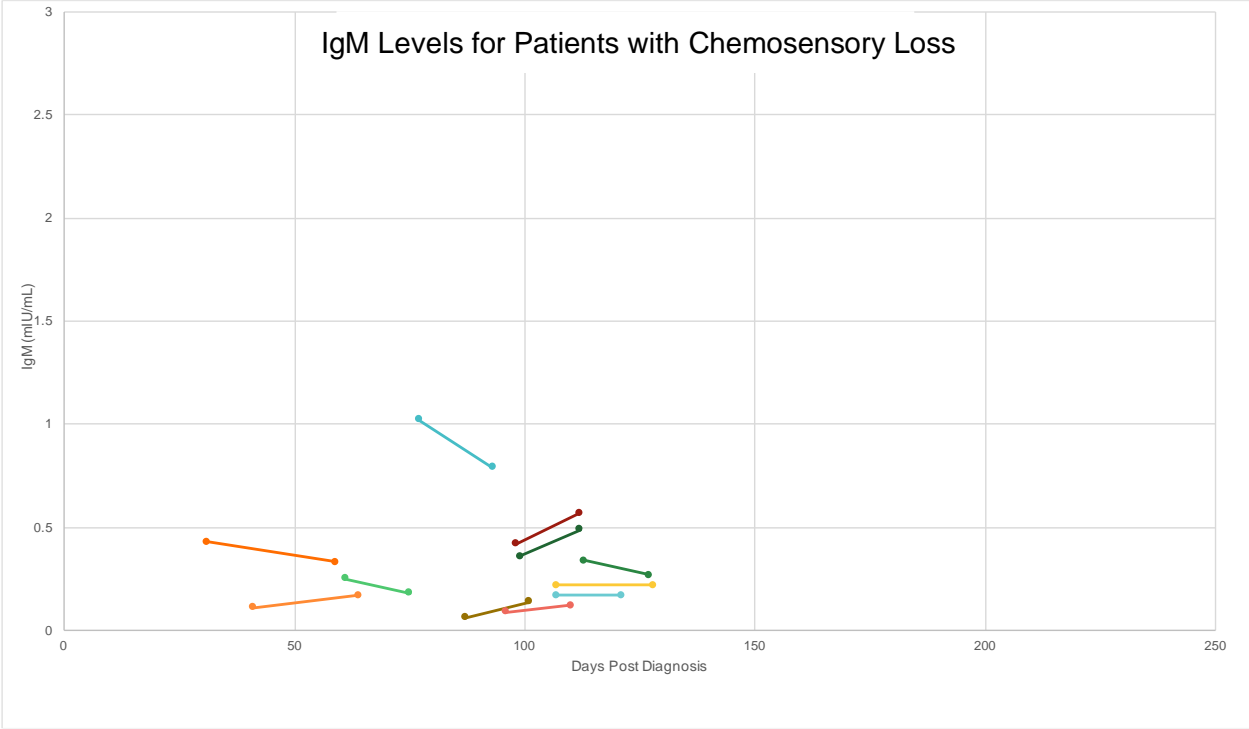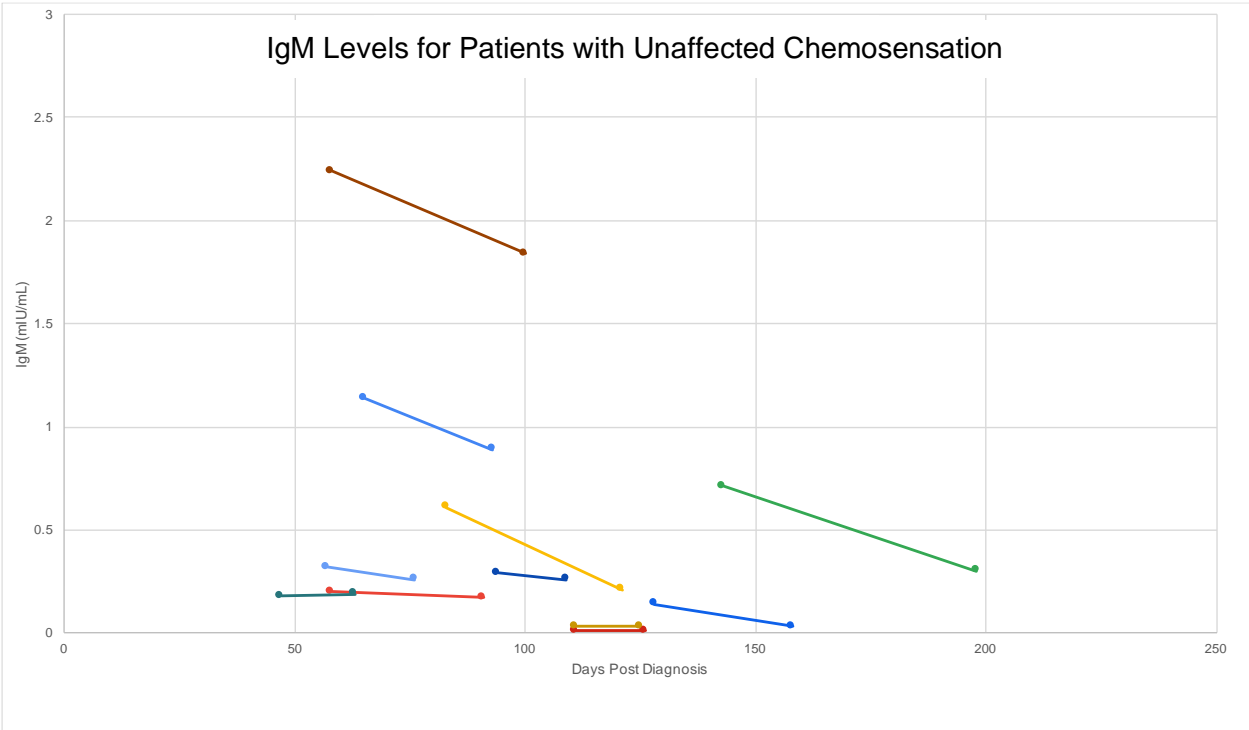307 2021 Feb;93(2):924-33.

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311 **FIGURES**
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Clinical Symptomatology in COVID-19 Patients

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315 *Figure 1*.
316 Clinical symptomatology in a case series of 22 COVID-19 patients.
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IgM Levels for Patients with Chemosensory Loss

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319 Panel A
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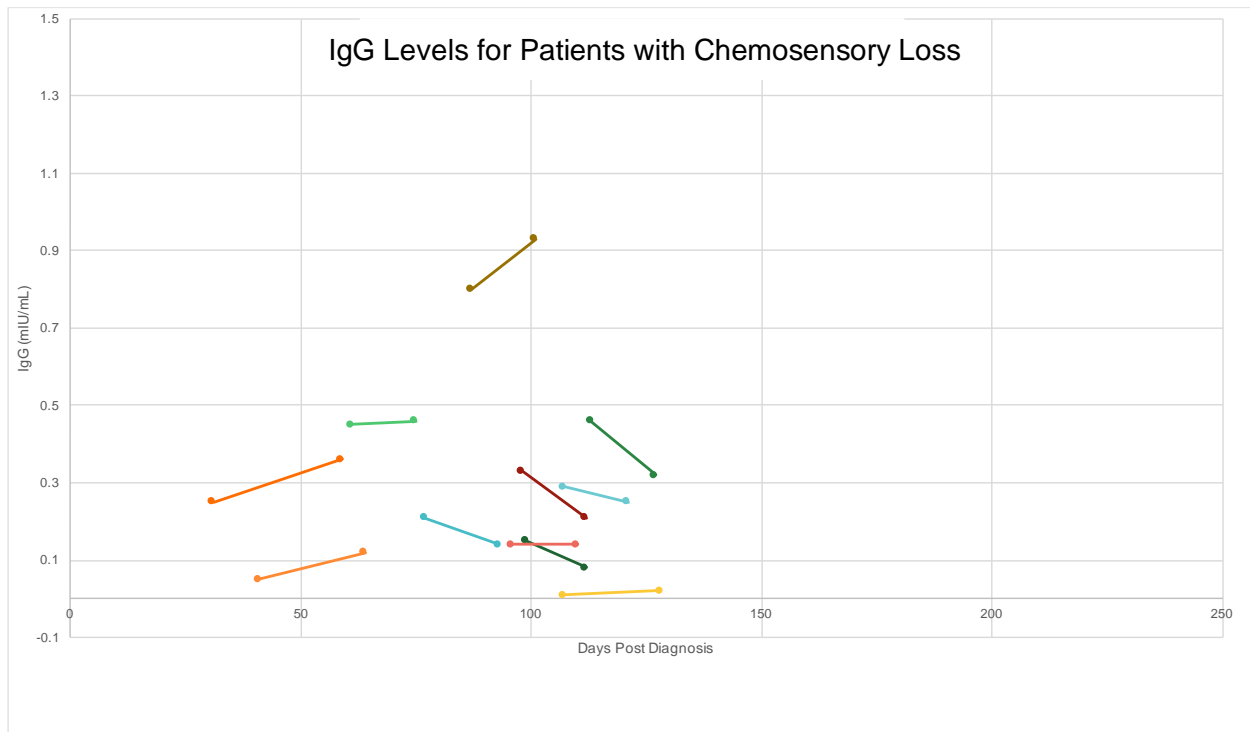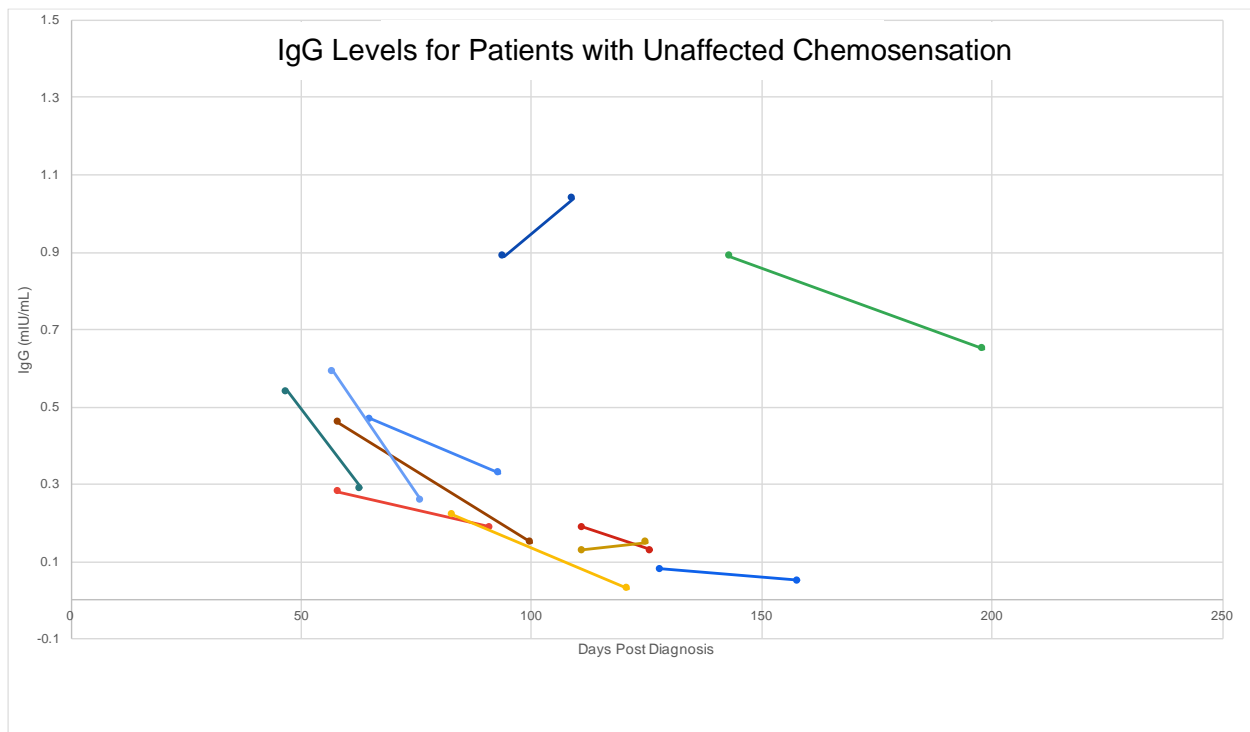IgM Levels for Patients with Unaffected Chemosensation

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322 Panel B
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324 *Figure 2*.
325 Quantitative and longitudinal IgM titers for individual patients.
326 Panel A – patients who experienced anosmia or ageusia (N=11).
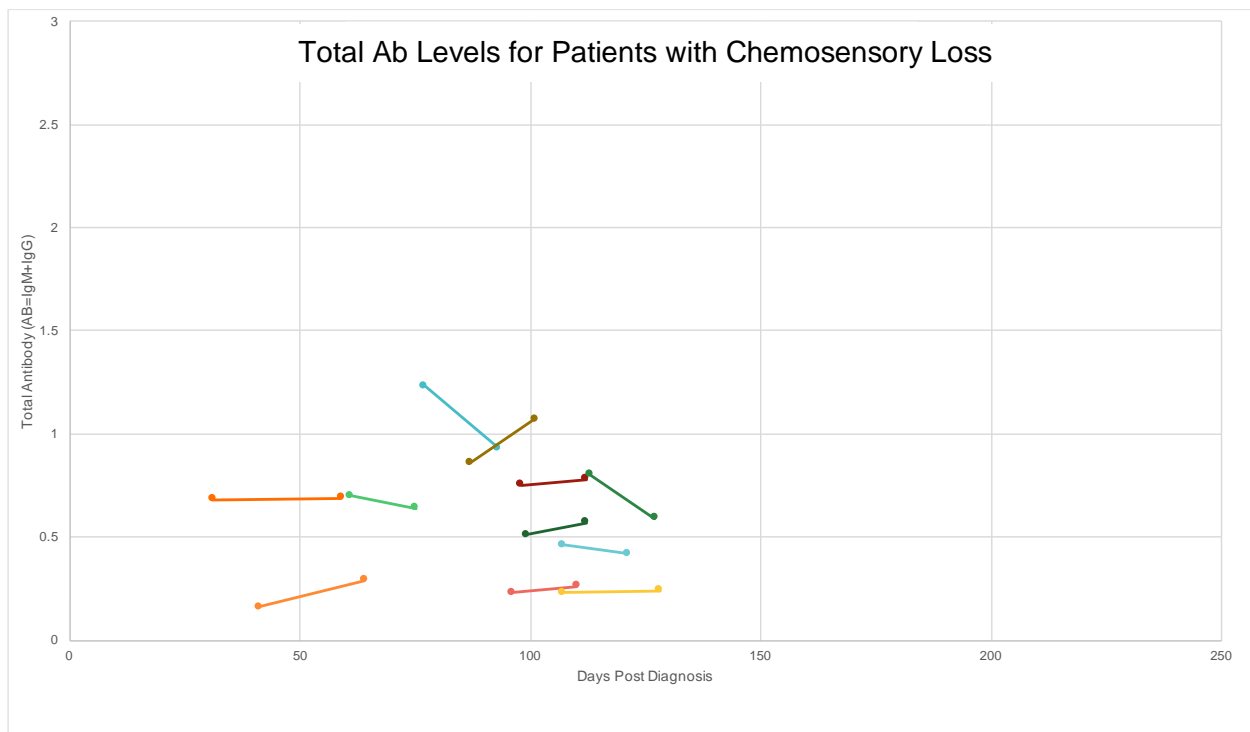327 Panel B – patients without chemosensory deficits (N=11).
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329
330   Panel A

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333   Panel B

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335   ***Figure 3***.
336   Quantitative and longitudinal IgG titers for individual patients.
337   Panel A – patients who experienced anosmia or ageusia (N=11).
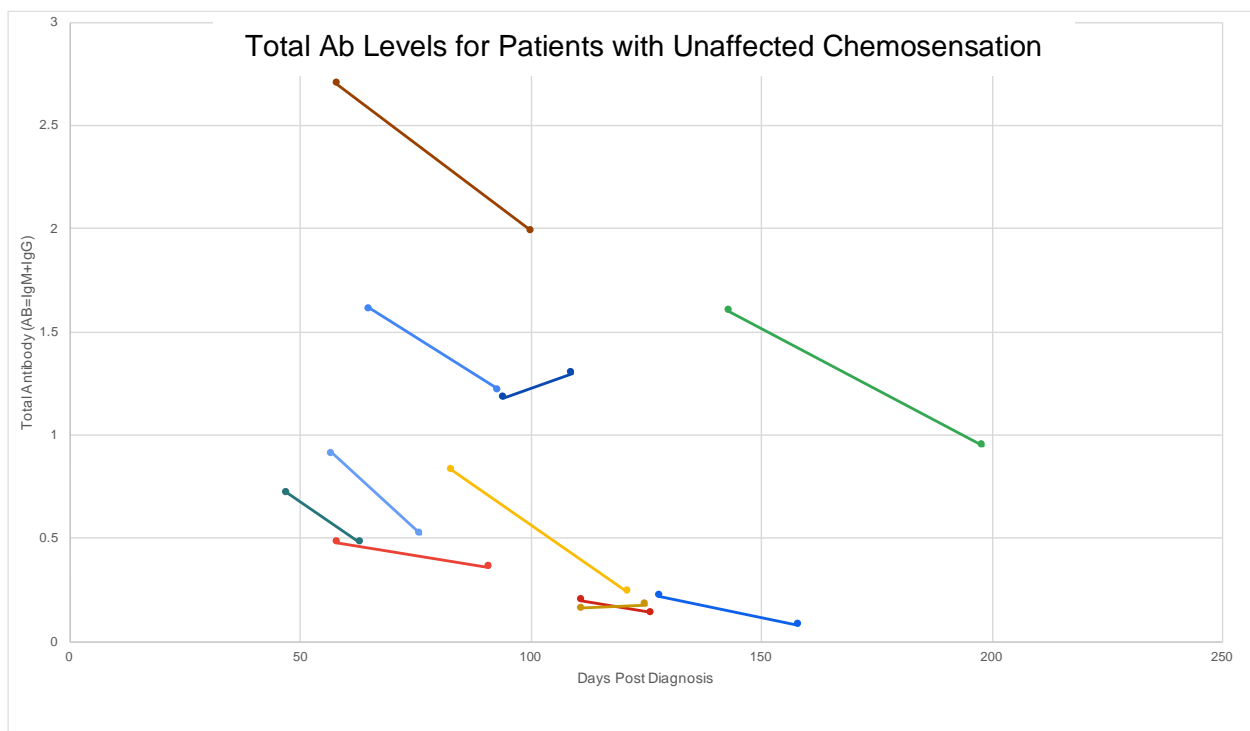338   Panel B – patients without chemosensory deficits (N=11).
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341 Panel A
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344 Panel B
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346 *Figure 4*.
347 Quantitative and longitudinal Total Antibody titers (IgM + IgG) for individual patients.
348 Panel A – patients who experienced anosmia or ageusia (N=11).
349 Panel B – patients without chemosensory deficits (N=11).