Gastrointestinal toxicities of immune checkpoint inhibitors: a multicenter retrospective analysis

Christine Shieh, Divya Chalikonda, Peter Block, Brianna Shinn, C. Andrew Kistler
Thomas Jefferson University Hospital, USA

Abstract

Background Immune checkpoint inhibitors are monoclonal antibodies that augment immune cell function and are used to treat malignancy. However, they may cause proinflammatory adverse events. This study investigated gastrointestinal (GI) adverse events associated with specific immune checkpoint inhibitors.

Methods Charts of patients aged >18 years with a solid tumor who underwent treatment with immune checkpoint inhibitors between 1st April 2011 and 1st August 2019 were reviewed for GI toxicities. Clinical data, including interventions, treatment duration and outcomes, were recorded.

Results One hundred patients were included in the study, of whom 22 experienced a GI adverse event directly attributable to immune checkpoint inhibitors. Transaminitis (9/22; 40.9%) and colitis (8/22; 36.3%) were most prevalent. The majority of events occurred within 4 cycles of treatment onset and were most prevalent with the nivolumab + ipilimumab combination (7/12; 58.3%). In 91% of cases (20/22), patients showed improvement or resolution of the event. Among the colitis cases, there was a significant difference (P=0.004) in recovery time between those who received infliximab and those who did not. Despite symptom resolution, only 7/22 were left on the same or part of the same treatment regimen.

Conclusions Most patients experienced their GI adverse events within 4 cycles of starting treatment, the most common being transaminitis and colitis. Nivolumab + ipilimumab dual therapy was most strongly associated with colitis. Most adverse events self-resolved, with infliximab being particularly helpful in improving colitis symptoms. However, most patients were unable to tolerate the same immunotherapy regimen and ultimately expired.

Keywords Oncology, checkpoint inhibitor, gastrointestinal, colitis

Ann Gastroenterol 2020; 33 (6): 1-7

Introduction

Immune checkpoint inhibitors (ICIs) make up a novel class of drug that has revolutionized the therapeutic approach to various malignancies and has consequently become increasingly common in daily oncology practice. These medications facilitate the inhibition of down-regulators of the immune system, thereby augmenting immune cell function via various pathways and eventually resulting in increased activation of T-cells [1,2]. Several monoclonal antibodies have been approved by the Food and Drug Administration for use in various malignancies; these include programmed cell death protein ligand inhibitors (PD-L1 inhibitors: atezolizumab, avelumab, durvalumab), programmed cell death protein inhibitors (PD-1 inhibitors: nivolumab, pembrolizumab), and cytotoxic T-lymphocyte associated antigen inhibitors (CTLA-4 inhibitors: ipilimumab, tremelimumab) [3].

By augmenting the activity of the immune system, ICIs can induce proinflammatory adverse events, termed immune-related adverse events (irAEs) [2,4]. Though irAEs can affect any organ, the endocrine glands, skin, gastrointestinal (GI) tract, and liver are most frequently implicated [2-4]. GI tract involvement is one of the most common types of irAEs, constituting about 30-50% of the total [5,6]. The exact pathophysiology of irAEs is not completely understood, but is thought to be a manifestation of a disruption of immune system homeostasis, resulting in altered T-cell and cytokine responses [4,5]. Studies thus far have shown that the organ
systems affected by ICIs vary based on the specific molecule targeted, whether PD-1, PD-L1 or CTLA-4 [2,5]. However, given their relatively recent increased use, the frequency with which various ICIs cause organ-specific AEs, particularly within the GI tract, has yet to be fully elucidated.

Beyond frequency, several aspects of irAEs remain unclear, including approach to treatment, treatment outcome and timeframe of presentation and resolution. In turn, the identification and management of irAEs have significant heterogeneity in practice, while a significant amount of research and data is still accumulating. GI and liver AEs frequently result in referrals to gastroenterologists and hepatologists. Typical GI irAEs noted include immune-mediated colitis (8-22%), hepatotoxicity (4-11%), pancreatitis (10-15%), and non-specific symptoms of nausea, vomiting and diarrhea (27-54%) [5-7]. Early recognition and suspicion for irAEs are critical to prevent detrimental clinical outcomes such as liver failure, toxic megacolon or bowel perforation. Therefore, we aimed to identify the GI irAEs associated with ICIs in this multi-center study and to describe the prevalence, management, and clinical outcomes of GI irAEs to further fill the current research void regarding these agents.

Patients and methods

Patients

The electronic health record (EHR) for Thomas Jefferson University Hospital and Methodist Hospital was queried to identify patients aged over 18 years with a solid tumor cancer who underwent treatment with ICIs between 1st April 2011 and 1st August 2019. Demographic data recorded included type and stage of cancer, Eastern Cooperative Oncology Group (ECOG) Performance Status, and smoking and alcohol use. Patients were not receiving concomitant active chemotherapy. Our protocol was approved by the institutional review board. Patient charts were screened for ICD-10 codes with the following GI AEs: colitis, hepatitis, pancreatitis, elevated bilirubin or transaminases, nausea, vomiting, diarrhea, and abdominal pain. These charts were reviewed for GI AEs attributed to the ICIs according to clinical documentation by the oncologist. Attributing AEs to immunotherapy was based on the patient’s symptoms, improvement upon discontinuation of the immunotherapy and/or treatment with steroids. Clinical data for each GI AE recorded included grading by the Common Terminology Criteria for Adverse Events scale, number of cycles prior to the GI AE, interventions, recovery time, and clinical outcomes.

Statistical analysis

Categorical and continuous variables were summarized using proportions and percentages. Mean ± standard deviation was calculated for continuous variables when possible. Univariate analysis was performed on recovery time.

Results

A total of 100 patients met the inclusion criteria and were screened for GI AEs while on ICIs. Patient characteristics and immunotherapy treatment are presented in Table 1. Seventy-five patients were functional, with ECOG scores of 0 or 1, while 85/100 had advanced stage cancer, either Stage III or IV. The most common cancers represented in our study were melanoma (41/100) and lung cancer (34/100).

Of the 100 patients, 22 experienced a GI AE closely attributed to the use of ICIs. These GI AEs were distributed as follows: 9/22 patients with transaminitis, 8/22 with colitis, 3/22 with unspecified nausea and diarrhea, and 2/22 with pancreatitis (Table 2). Patients received an average of 4 cycles of immunotherapy prior to experiencing side-effects. For the 2 most common side-effects seen, transaminitis was associated with nivolumab use in 6/9 cases and colitis was associated with the dual regimen of nivolumab/ ipilimumab in 5/8 cases (Fig. 1). In 91% of cases (20/22), patients had improvement or resolution of the GI AE. There was no significant difference in recovery time between transaminitis and colitis (P=0.38); the mean recovery time was 43±36 days. However, among the colitis cases, there was a significant difference (P=0.004) in recovery time between those who received infliximab (IFX) (10.75±11.3 days) and those who did not (63.75±16.3 days). Despite resolution of the GI symptoms, only 7/22 were maintained on the same or part of the same treatment regimen. Ultimately, the mortality rate for this group of patients experiencing GI AE on immunotherapy was 41% (9/22), although the GI AE were not necessarily the cause of death. Of the deaths, only one involved...
The distribution of side effects for each chemotherapy regimen is shown in Fig. 2. The majority of patients on nivolumab + ipilimumab (58.3%; 7/12) experienced a GI AE. Among those on nivolumab monotherapy, 26.1% (6/23) of patients had a GI AE, while for those on pembrolizumab monotherapy 11.9% (5/42) had a GI AE. The numbers of cases for the other immune checkpoint inhibitor regimens were small, so other patterns were difficult to appreciate.

Discussion

As an increasing number of patients are treated with ICIs, the incidence and relevance of GI AEs are also increasing. In our study of 100 patients, 22% had documented GI AEs. This is less than the current reported range of 30-50%; the difference may be due to our limited cohort size and the constraint of relying on chart documentation for data [8]. Of the GI AEs, transaminitis (41%) and colitis (36.3%) were most commonly encountered in our cohort. In the past, colitis has been described as the most common GI side-effect after the administration of ICIs, while hepatitis has only been observed in <5% of cases [8,9]. This discrepancy in prevalence may be attributed to the asymptomatic nature of some patients with elevated transaminases whose AEs therefore go undetected.

In general, CTLA-4 inhibitors such as ipilimumab and tremelimumab have higher rates of irAEs than PD-1/L1 inhibitors such as nivolumab and pembrolizumab, with 90% occurring in the former and 70% occurring in the latter [2,10]. Despite this, in our cohort transaminitis was most commonly seen with the PD-1/L1 inhibitor nivolumab. Colitis was most commonly experienced by patients on both nivolumab and ipilimumab. It is unclear if this observation is due to the use of ipilimumab in the dual-therapy regimen or to the additive effect of nivolumab. Diarrhea has been reported in up to 30% of patients following CTLA-4 therapy, with 5% having severe colitis [4,11]. Only <4% of diarrhea cases have been attributed to anti-PD-L1 therapy [4,12]. However, Bajwa et al conducted a meta-analysis review of all case reports/case series in the PubMed database between 2016 and 2018 and found 14 cases of colitis, over half of which were attributed to nivolumab [6]. The prevalence of colitis on dual therapy regimen in our study may therefore be due to the additive side-effects of both drugs together. Soldatos et al conducted a large retrospective study of irAEs after the use of nivolumab alone, ipilimumab alone, or the 2 together to treat melanoma, and found that the combination of the 2 led to an expanded toxicological profile that included the side-effect profile of both agents, with higher incidences of colitis, rash, and pyrexia [13]. Patients on this combination regimen should therefore be observed carefully for early signs of worsening diarrhea.

Nearly all of our patients had improvement in their irAEs. The majority of transaminitis and pancreatitis cases were improved by holding immunotherapy and observing. Two of the higher-grade cases of transaminitis received steroids, while both cases of high-grade pancreatitis did not and still improved. The 8 cases of colitis received a course of prednisone. Of the 8 cases, 4 received IFX and had a notably quicker recovery rate in terms of symptomatology.

Table 1 Patient characteristics

| Parameter                        | Value |
|----------------------------------|-------|
| Age (±SD)                        |       |
| Male                             | 66.0±12.4 |
| Female                           | 67.9±10.2 |
| Sex                              |       |
| Male                             | 60    |
| Female                           | 40    |
| Type of cancer                   | # of cases (of 100) |
| Melanoma                         | 7     |
| Eye                              | 6     |
| Skin, BRAF mut                   | 18    |
| Skin, BRAF wt                    | 10    |
| Lung                             |       |
| Head and neck                    | 5     |
| Prostate                         | 3     |
| Renal                            | 4     |
| Breast                           | 2     |
| Hodgkin’s                        | 2     |
| Uterine                          | 1     |
| Vulvar                           | 1     |
| Bladder                          | 1     |
| ECOG                             |       |
| 0                                | 36    |
| 1                                | 39    |
| 2                                | 9     |
| 3                                | 5     |
| 4                                | 1     |
| Stage                            |       |
| I                                | 4     |
| II                               | 9     |
| III                              | 18    |
| IV                               | 67    |
| Unclear                          | 2     |
| Social factors                   |       |
| Former smoker                    | 44    |
| Active smoker                    | 8     |
| Active alcohol use               | 40    |
| Underlying GI/autoimmune comorbidities | 1 collagenous colitis |
| Immunotherapy regimens           |       |
| Pembrolizumab                    | 42    |
| Nivolumab                        | 33    |
| Nivolumab + ipilimumab           | 12    |
| Nivolumab + brentuximab          | 1     |
| Durvalumab                       | 5     |
| Atezolizumab                     | 3     |
| Ipilimumab                       | 4     |

Mut, mutant; Wt, wild type; ECOG, Eastern Cooperative Oncology Group; GI, gastrointestinal
| GI AE       | Symptoms                                      | Significant lab or imaging abnormalities | Grade (CTCAE) | # of cycles of IT | IT                        | GI AE Treatment                                                                                       | Treatment outcome | Recovery time | Clinical Outcome                                                                 |
|------------|----------------------------------------------|------------------------------------------|---------------|-------------------|-------------------------|-----------------------------------------------------------------------------------------------------|-------------------|---------------|----------------------------------------------------------------------------------|
| transaminitis | asymptomatic                                 | AST 116 U/L ALT 247 U/L                  | 1             | 3                 | nivolumab/ ipilimumab   | held IT                                                             | resolved           | 14 days        | continued on nivolumab only                                                      |
| transaminitis | asymptomatic                                 | AST 77 U/L ALT 52 U/L                    | 1             | 3                 | nivolumab               | held IT                                                             | resolved           | 30 days        | continued treatment                                                             |
| transaminitis | asymptomatic                                 | AST 131 U/L ALT 105 U/L                  | 1             | 3                 | pembrolizumab           | held IT                                                             | resolved           | 7 days          | not resumed due to other side effects, eventually died                           |
| transaminitis | fever, headache                              | AST 540 U/L ALT 445 U/L                  | 4             | 2                 | nivolumab/ ipilimumab   | held IT prednisone IV 1 mg/kg with PO taper over 4 months          | resolved           | 4 months        | not resumed on current treatment, eventually died                               |
| transaminitis | asymptomatic                                 | AST 84 U/L ALT 153 U/L                   | 1             | 2                 | nivolumab               | held IT                                                             | resolved           | 3 months        | continued treatment, eventually died                                            |
| transaminitis | abdominal pain                               | AST 208 U/L ALT 76 U/L ALK 900 U/L       | 3             | 1                 | nivolumab               | held IT                                                             | resolved           | 1 week          | died within 1 week                                                              |
| transaminitis | asymptomatic                                 | AST 78 U/L ALT 78 U/L                    | 1             | 5                 | nivolumab               | held IT                                                             | resolved           | 45 days         | continued treatment                                                             |
| transaminitis | abdominal pain                               | AST 195 U/L ALT 150 U/L total bilirubin: 10.5 mg/dl direct bilirubin: 8.6 mg/dl | 3             | 3                 | ipilimumab              | prednisone IV 1 mg/kg ×1 then PO 40 mg BID                          | hospice            |                |                                                                                  |
| transaminitis | asymptomatic                                 | AST 74 U/L ALT 52 U/L ALK 1100 U/L total bilirubin: 4.7 mg/dl | 1             | 2                 | pembrolizumab           | prednisone 70 mg with taper to 10 mg over 2 months                  | improved           | 2 mo            | plan for chemoembolization, eventually died                                       |
| colitis     | diarrhea                                      | colitis                                  | 2             | 3                 | nivolumab/ ipilimumab   | prednisone 80 mg × 5d taper to 15 mg over 5 months                  | resolved           | 45 days         | not resumed on current treatment                                                |
| colitis     | diarrhea                                      | colitis                                  | 2             | 5                 | ipilimumab              | prednisone 50 mg × 3 d with taper to 5 mg over months budesonide 3 mg TID with taper to BID | resolved to baseline (has collagenous colitis) | 90 days        | got 1 more infusion, eventually died                                            |
| colitis     | pain, diarrhea, bandemia                      | colitis                                  | 4             | 2                 | nivolumab/ ipilimumab   | prednisone IV then PO 60 mg BID taper to 20 mg over months Infliximab 16 mg/kg ×1 | resolved           | 7 days          | got 1 more infusion but developed other side effects                           |
| colitis     | diarrhea                                      | colitis                                  | 1             | 2                 | nivolumab/ ipilimumab   | prednisone 10 mg                                                   | resolved           | 60 days         | not resumed on current treatment                                                |

(Contd..)
| GI AE | Symptoms | Significant lab or imaging abnormalities | Grade (CTCAE) | # of cycles of IT | IT | GI AE Treatment | Treatment outcome | Recovery time | Clinical Outcome |
|-------|----------|-----------------------------------------|---------------|-----------------|----|----------------|------------------|--------------|------------------|
| colitis | diarrhea | colitis                                 | 2             | 2               | nivolumab/ipilimumab | prednisone 40 mg BID Infliximab 5 mg/kg ×2 | resolved | 30 days | hospice |
| colitis | diarrhea | colitis                                 | 2             | 29 for nivolumab/6 for ipilimumab | nivolumab/ipilimumab | prednisone 1 mg/kg IV tapered to 80 mg PO then off in 1 month Infliximab 5 mg/kg ×1 | resolved | 5 days | not resumed on current treatment |
| colitis | diarrhea | colitis                                 | 2             | 2               | pembrolizumab | prednisone IV 1 mg/kg × 3d with PO taper for 1 month Infliximab 5 mg/kg ×1 | resolved | 1 day | not resumed on current treatment |
| colitis | diarrhea | colitis                                 | 3             | 10              | nivolumab | prednisone 10 mg | improved | 4 months on Creon now | not resumed on current treatment |
| pancreatitis | flank pain | lipase 1485 U/L absolute eosinophil count 756 imaging findings | 3             | 2               | pembrolizumab | held IT | resolved | 2 months | dose reduced to half |
| pancreatitis | abdominal pain | lipase 980 U/L imaging findings | 4             | 19              | durvalumab | held IT | resolved | 4 months on Creon now | not resumed on current treatment |
| non-specific nausea, cramps, diarrhea | No significant abnormalities | 1             | 3               | nivolumab/brentuximab | supportive care | continued treatment |
| non-specific diarrhea | No significant abnormalities | 2             | 9               | nivolumab | held IT | resolved | 120 days | switched to another agent because of disease progression |
| non-specific diarrhea | No significant abnormalities | 1             | 1               | pembrolizumab | supportive care | continued treatment |

CTCAE, common terminology criteria for adverse events; GI, gastrointestinal; AE, adverse event; IT, immunotherapy; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALK, alkaline phosphatase.
Figure 2 Distribution of side effects for each chemotherapy regimen. This graph depicts the relative number of adverse events related to the gastrointestinal tract for each immunotherapy regimen. Notably, the majority of patients on combo therapy with nivolumab + ipilimumab had GI AEs

GI, gastrointestinal; AE, adverse event

compared to those who did not receive IFX. The use of IFX in treating irAEs is suggested as second line after patients do not respond to steroids [14]. Most patients do respond well to IFX [14]. Given the quick recovery rate of patients on IFX—on the order of 10 days in our study—this therapy may be used in an attempt to avoid the prolonged use of steroids, which leads to higher infection rates [15]. Although IFX offers an alternative to prolonged steroid use, its use also comes with risks. Patients being considered for IFX must have up-to-date screening for hepatitis B as well as tuberculosis [16]. In addition, patients with severe heart failure or concurrent elevated serum aminotransferases may not be ideal candidates for IFX [16].

While most patients had resolution of their GI AEs, only 7/22 were maintained on their original treatment regimen, in some cases at a lower dosage. This statistic is marred by the fact that some patients expired prior to engaging in conversation about future treatment. Notably, no patients with colitis were able to continue on their current regimen, because of the physician’s clinical judgement, intolerance to further sessions, or death. In general, severe colitis occurs in <10% of the cases and ICIs may be restarted up until grade 3 colitis if the benefits outweigh the risks [5,16,17]. The more conservative approach in our cohort may be in line with the documented fatalities associated directly with GI AEs. A meta-analysis that represented the largest evaluation of fatal toxic effects of ICIs reported colitis to be the most frequent cause of anti-CTLA-4 toxicity death, while anti-PD1 was more often associated with hepatitis [18].

Our cohort of patients experiencing GI AE on immunotherapy had a high mortality rate at 41%, despite having high ECOG performance status between 0 and 1, but no deaths were directly recorded to be attributed to the GI AE event, although one patient was hospitalized for GI AE and discharged directly home to hospice. In general, fatal toxicities directly attributed to GI AE are rare [15], but many of our patients expired after discontinuing their immunotherapy and were in the process of evaluating other treatment options. Given the high mortality rate of patients with GI AE findings in our cohort, it is important to screen and treat GI AEs early on, with the hope of either continuing on the current treatment regimen or planning ahead for secondary options.

Our study is limited by the number of cases of ICI GI AEs, as well as the lack of complete data recorded in the EHR. Future work will be directed towards expanding the cohort so that more data can be collected regarding other ICI regimens of which we only had a few cases (i.e., atezolizumab, durvalumab). Overall, our study depicts colitis and transaminitis to be the most prevalent GI AEs encountered in patients on immunotherapy. Notably, the combination of nivolumab with ipilimumab was highly associated with colitis. Cases of GI AEs do resolve with proper treatment and care, and IFX is a particularly effective agent for quickly improving symptomatology in colitis. Despite resolution, most patients are unable to continue on the current treatment regimen and still have a high mortality rate, though deaths are not directly attributable to GI AEs.

**Summary Box**

**What is already known:**

- Gastrointestinal toxicities are a common side-effect of immunotherapy, with colitis being charted as the most common side-effect
- Steroids and sometimes anti-inflammatory immunomodulators are used to treat gastrointestinal adverse events that are high grade on the Common Terminology Criteria for Adverse Events scale
- Fatalities resulting directly from these gastrointestinal adverse events are uncommon

**What the new findings are:**

- Colitis is a particularly common side-effect in patients on dual therapy with nivolumab/ipilimumab
- The majority of patients are not maintained on their current immunotherapy regimen, leading to a search for alternatives
- While most cases of gastrointestinal adverse events due to cancer immunotherapy achieve resolution, the overall end mortality rate of patients experiencing these events is still quite high
References

1. Azoury SC, Straughan DM, Shukla V. Immune checkpoint inhibitors for cancer therapy: clinical efficacy and safety. *Curr Cancer Drug Targets* 2015;15:452-462.
2. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *N Engl J Med* 2018;378:158-168.
3. Pento, JT. Monoclonal antibodies for the treatment of cancer. *Anticancer Res* 2017;37:5935-5939.
4. Yoest, JM. Clinical features, predictive correlates, and pathophysiology of immune-related adverse events in immune checkpoint inhibitor treatments in cancer: a short review. *Immunotargets Ther* 2017;6:73-82.
5. Som A, Mandalia R, Alsaadi D, et al. Immune checkpoint inhibitor-induced colitis: a comprehensive review. *World J Clin Cases* 2019;7:405-418.
6. Bajwa R, Cheema A, Khan T, et al. Adverse effects of immune checkpoint inhibitors (programmed death-1 inhibitors and cytotoxic T-lymphocyte-associated protein-4 inhibitors): results of a retrospective study. *J Clin Med Res* 2019;11:225-236.
7. Rajha E, Chaftari P, Kamal M, et al. Gastrointestinal adverse events associated with immune checkpoint inhibitor therapy. *Gastroenterol Rep (Oxf)*. 2020;8:25-30.
8. Liu YH, Zang XY, Wang JC, et al. Diagnosis and management of immune related adverse events (irAEs) in cancer immunotherapy. *Biomed Pharmacotherapy* 2019;120:109437.
9. Paydas, S. Gastrointestinal side effects of immune checkpoint inhibitors: new topic for gastroncologists. *Hematol Med Oncol* 2019;4:1-9.

10. Baxi S, Yang A, Gennarelli RL, et al. Immune-related adverse events for anti-PD-1 and anti-PD-L1 drugs: Systematic review and meta-analysis. *BMJ* 2018;360:k793.
11. Hodi FS, O’Day SJ, McDermott DE, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med* 2010;363:711-723.
12. Wang PF, Chen Y, Song SY, et al. Immune-related adverse events associated with Anti-PD-1/PD-L1 treatment for malignancies: a meta-analysis. *Front Pharmacol* 2017;18:730.
13. Soldatos TG, Dimitrakopoulou-Strauss A, Larribere L, et al. Retrospective side effect profiling of metastatic melanoma combination therapy ipilimumab-nivolumab using adverse event data. *Diagnostics* 2018;8:E76.
14. Johnston RL, Lutzky J, Chodhry A, et al. Cytotoxic T-lymphocyte-associated antigen 4 antibody-induced colitis and its management with infliximab. *Dig Dis Sci* 2008;54:2538.
15. Wang DY, Salem JE, Cohen JV, et al. Fatal toxic effects associated with immune checkpoint inhibitors: A systematic review and meta-analysis. *JAMA Oncol* 2018;4:1721-1728.
16. Bristol-Myers Squibb Company. Opdivo immune-mediated adverse reactions management guide. Available from: https://www.opdivohcp.com/assets/commercial/us/opdivo-hcp-pan-tumor/en/pdf/Immune_Mediated_Adverse_Management_Guide.pdf [Accessed 7 September 2020].
17. Prieux-Klotz C, Dior M, Damotte D, et al. Immune checkpoint inhibitor-induced colitis: diagnosis and management. *Target Oncol* 2017;12:301-308.
18. Jiang Y, Zhang N, Pang H, et al. Risk and incidence of fatal adverse events associated with immune checkpoint inhibitors: a systematic review and meta-analysis. *Ther Clin Risk Manaj* 2019;15:293-302.